

How to order an East-West thaw

The word in Washington is that the administration is about to try again for better scientific relations with the Soviet Union. The goal should be to put any new initiative on a permanent foundation.

THE tale of how Sir Humphry Davy went on a tour of mainland European laboratories at the height of the Napoleonic Wars is widely regarded as a proof that the international character of science was then more highly regarded than now. That Davy, to whom uxoriousness came late, was also on his honeymoon, makes the journey all the more remarkable. Certainly things are different now, as the near-collapse of scientific relations between the West and the Soviet Union demonstrates all too clearly. Part of the explanation is that research is now recognized to bear on the immediate strategic interests of governments. It also matters that the scientific community everywhere is now dependent on governments for financial support, and thus constrained. But, since the early nineteenth century, procedures by which the movement of people from one country to another is regulated have also been formalized and tightened, partly in response to the flourishing of the technology of travel but also, one suspects, often for its own sake. The time, however, has come to ask that the present parlous condition of East-West relations should not be allowed to continue as it is.

The issue is urgent not merely in its own right but because, by some accounts, President Reagan was earlier this week about to advocate in public a renewed attempt to foster scientific relations between the United States and the Soviet Union. Many outside the Soviet Union will be surprised by this development, not least Dr Frank Press, president of the National Academy of Sciences in Washington. Earlier this summer, Dr Press announced his intention of travelling to the Soviet Union to breathe new life into the largely defunct exchange programme with the Soviet Union. Dr George Keyworth, President Reagan's science adviser, said at the time (see *Nature* 10 May, p.105) that he was "surprised" at this development. But then, with anxiety about the condition of Andrei Sakharov mounting, Dr Press put off his visit. Other academies in the West have responded similarly to recent developments. In London for example, the Royal Society declined a formal invitation to last week's conference in Moscow to celebrate the twenty-fifth anniversary of the Institute of Biochemistry, now run by Academician Y. A. Ovchinnikov. President François Mitterrand, as the world now knows, has chosen a more direct way of dealing with a delicate problem of diplomatic etiquette; last week, in Moscow to seek some basis for continuing East-West negotiations on arms control, he spoke up about Western anxiety about Andrei Sakharov — and had his remarks on the subject censored from *Pravda's* otherwise complete account of what he had to say.

A sorry tale

The recent deterioration of East-West relations is so familiar and depressing that it hardly bears recitation. Underlying the sorry tale is a sequence of calamitous developments that can be counted as misunderstandings only by the exercise of the utmost charity. The Soviet deployment dating from the mid-1970s of SS20 missiles within striking range of Western Europe broke the spirit but not the letter of the SALT II agreement on strategic arms. Soviet signature in 1975 of the Helsinki agreement within the framework of the Conference on Security and Cooperation in Europe, which among other things provided for a measure of liberality in governments' dealings with their people, did not prevent the arrest of members of the Helsinki monitoring group

set up in Moscow. The Soviet intervention in Afghanistan at the end of 1979, ostensibly at the invitation of the government in Kabul, was a more serious setback; the then President Carter gave that as his reason for not sending the SALT II treaty to the Senate for ratification, although by then it was already probable that he would have been given a dusty answer.

Serious interruptions of agreements on the East-West exchange of scientists date, however, from this time. So do the efforts in the United States to restrain the "haemorrhage" of Western technology to the East, with the variously grudging consent of Western Europe and Japan. The exile (to Gor'kii) of Andrei Sakharov, and his subsequent ill-treatment, seems to many to have been the last straw.

Principles

Neither East nor West has dealt rationally with the several sorry events in this unpleasant history. The principles that allowed Humphry Davy to go gallivanting in mainland Europe when France was at war with most other European states seem to have been forgotten. Put simply, they are these. While national governments have necessarily become the chief sources of support for scientific research in all fields, governments have not by that circumstance bought the scientific enterprise, which remains the collective property of its practitioners. And while professional people owe their national governments allegiance of the kind required by the civil law (which varies from place to place), they also owe their profession the duty of acknowledging the international character of what they do. East-West rivalry in military development and in the commercial exploitation of technology, unavoidable at some level, paradoxically must coexist with East-West interdependence in basic science. That is the simple truth that the Pentagon's planners seem only with difficulty to have appreciated in their schemes for restricting visits for foreigners to US laboratories. Exactly the same principle works in the opposite direction, Moscow's puzzlement at the West's indignation over Sakharov notwithstanding.

The translation of these principles into practice should not be as difficult as it is often made to seem by the self-contradictions of governments and the hesitancy of professional people.

If the Soviet Union should send its military forces into Afghanistan on a trumped-up excuse, shoot down a Korean civil aircraft or walk out of negotiations on arms control, that is no reason why the East-West migration of scientists should be constrained. But, naturally, visitors in one direction or the other, being adult electors as well as scientists, will or should be unrestrained in saying what they find objectionable in the policies of their hosts' government. Because of the belief, common in the West and justifiable, that Soviet citizens are not always allowed to judge for themselves what is the truth about political developments, it is inconsistent that exchange programmes should be cancelled or reduced at times when, in a small way, they might contribute precisely to the kind of enlightenment that is supposedly lacking. It is, however, important that these opinions should be advanced only in ways consistent with local civil law and in a manner that does not impede the chief purpose of a visit (which usually, in practice, means privately and not in public). Arguments about the relative durability of communism and capitalism, often entertaining, are irrelevant.



Indignation about the ways in which scientists are dealt with can be accommodated within the framework of seemingly East-West relations only with greater difficulty. The Soviet Government's justification of its treatment of Sakharov is logical enough. That a senior physicist, with such important contributions to the Soviet nuclear weapons programme to his credit, should have lapsed into such outspoken criticism of Soviet military and social policies, is regarded as disloyalty bordering on treachery. That view is consistent with the Leninist adaptation to modern times of the feudal Russian doctrine that people are the property of the state. (Soviet apologists for Sakharov's treatment are forever saying that he remains a member of the Soviet academy, with the appropriate salary, but that is beside the point.) What the Soviet Union appears not to understand is the offence which has been given in the West by its treatment of a distinguished if now dissident scientist which, while falling short of outright imprisonment, deprives the scientific community of such contributions as he may still have to make. (It is, on the other hand, unreasonable that Sakharov and his wife should appear to be asking, in a state whose civil laws restrict the freedom of people to move elsewhere — Helsinki notwithstanding — to be allowed medical treatment in the West.)

President Mitterrand seems to have sensed what these circumstances require with his statement in Moscow last week. There is every reason why lesser mortals should, in lesser ways, follow his example. The case is simply that it is a shabby way to treat a distinguished scientist, however troublesome he may have become, to pack him off to a provincial city out of touch with work, friends and colleagues. To say this when asked, and to volunteer the opinion should opportunity arise, need not cause rancour and may in the long run help Sakharov or at least the principles of civility. Better to seize the opportunity than to spurn it, either from embarrassment or in the belief that huffy silence will miraculously be more effective.

That is the most tricky part of the West's case for restoring East-West scientific relations. Let us hope that President Reagan's rumoured intentions materialize — and that the Soviet Union responds positively. But this time, at the umpteenth time of blowing hot, it is also important that the effort should be seriously intended. Nothing so permanently damages East-West relations as a set of formally negotiated agreements that is then vicariously abandoned because of some unrelated happening. So it would be useful to wring from the US Administration an undertaking that if East-West relations are now to be improved, there will be an understanding that they will not in future be put at hazard for political reasons having nothing to do with science. Although the US State Department has been quietly pragmatic in its administration of US-Soviet collaborations even in the past few difficult years, the simplest way of making sure that programmes now agreed continue is to delegate responsibility for them entirely to the National Academy in Washington which, with egg on its face since Dr Press blew hot, then cold, may be needing a little reassurance. □

New exams for old

Britain's plan to reorganize school examinations is sensible, but should be taken further.

THE British Secretary of State for Education and Science, Sir Keith Joseph, last week found himself the unaccustomed object of extravagant praise from all quarters of the British educational establishment. The occasion for this achievement, all the more remarkable in the atmosphere of ill-feeling left by strikes by teachers (over pay) in the past few weeks, was Sir Keith's decision finally to end the archaic and unfair two-tier system of examinations for 16-year-olds. For the past thirty-five years, there has been the "O" (for "ordinary") level of the General Certificate of Education (GCE) and, for twenty years, the Certificate of Secondary Education (CSE) for generally less able students. The notion that the two systems should be merged has been promoted for almost as long as they have co-existed. Five

years ago, the present government shelved a plan to merge the systems on the grounds that what it called "standards" would be undermined. Sir Keith's conversion to the educationalists' cause is belated, but none the less welcome on that account. The parable of the prodigal son refers. Will the lessons that have been learned, painfully it appears, now be applied elsewhere in the British educational system?

Sir Keith Joseph's scheme will by 1988 do away with the present jungle of 20 separate examination boards administering an astonishing 19,000 syllabuses for 16-year-olds. The confusion they have entailed has brought the whole examination system into disrepute: it is widely known that (for whatever reasons) the standards of some boards are substantially lower than those of others, even when the qualifications awarded are nominally the same. The boards will now be brought together into five groups to administer a single General Certificate of Secondary Education. Examinations under the new scheme will incorporate differentiated papers or questions that will be assessed to nationally-agreed criteria, thus making them applicable over a much wider ability range than the existing examinations. Students will not therefore have to choose — or, more often, be pushed — between examinations to which, for reasons of parental pride or teachers' convenience, they are not suited. National grade-related criteria now under development will in principle allow a more objective interpretation of examination results — and are indeed essential if the merger is to succeed. In a skilful compromise that seems to have avoided the inevitable accusation that a change will result in lowered standards, Sir Keith has been able to give an assurance that the performance represented by the three highest grades of the set of seven grades in the new examination will correspond to those of the old "O" level.

Why has this apparently obvious rationalization taken so long to decide? The British Government has consistently made clear its position that any integration of GCEs and CSEs would have to wait upon an acceptable agreement on national criteria for syllabuses and assessment procedures, and much effort has been expended towards this end by the examinations boards. Despite a few minor hiccups, such as the minister's reluctance at the outset to accept that a physics examination should include some questions on social and economic issues, the draft criteria have won reasonably wide support. (Even so, its draft criteria for biology at 16 includes no explicit reference to the theory of evolution.) What has been achieved shows that a national consensus on core subject content is feasible.

So will Sir Keith stop now that he has brought about his thoroughgoing revision of the national system of examinations at 16? The existing advanced level examinations ("A" levels), taken by those who choose to study until 18, are just as varied and just as much in need of revision, although their faults are different. In an effort to achieve more uniformity of content, nine examinations boards offering "A" levels have put out a document called *Common Cores at Advanced Level*, but this represents little more than the intersection of the separate syllabuses. And the boards have not yet been able to agree on even this much, with one or other of them declining to accept such-and-such a topic for inclusion. Although the notion that the existing "A" level examinations provide an education that is too narrow for the important years between 16 and 18 is widely accepted, yet, in the present state of anarchy, it is hard to see where a sensible revision in the direction of more generality could start.

The simplest solution would be to abolish this second tier of examinations. But how then would the universities know which school-leavers are best-suited to be students? That would be the academics' cry. There are two answers: provide a university entrance examination (as, for example, in the United States) and abolish the need for school specialization by not expecting most undergraduates to scamper through a first-degree course in just three years. For most British school-leavers who do not go on to further education, some of the time and effort that would be saved by the elimination of over-specialized teaching in secondary schools could be usefully spent in teaching other modern skills, from driving cars to operating computers. □

US space station

NASA negotiates on several fronts

Washington

THE National Aeronautics and Space Administration (NASA) is bracing itself for a complex series of negotiations with Europe, Congress and the space science community before it finalizes plans to build the \$8,000 million manned space station called for by President Reagan in his State of the Union message earlier this year.

In Washington last week, a delegation from the European Space Agency (ESA) settled down for what is expected to be a difficult round of talks with NASA about the cost and scope of European participation. NASA, under pressure from Congress and the Office of Management and Budget (OMB) to keep the costs of the station as low as possible, hopes to persuade foreign countries to pay up to 30 per cent of the station's cost.

But the Europeans, who are likely to account for between 15 and 20 per cent of the foreign contribution, are expected to drive a hard bargain.

At a meeting in Naples earlier this month, ESA officials said their member governments wanted to be more than subcontractors; they would be willing to join the project only as full partners able to garner the expertise necessary to launch a station of their own, perhaps a decade or so after the US station is launched in 1992. And to avoid repeating their mistakes with Spacelab, they are insisting on guarantees of equal access to the space station and the right to exploit it commercially.

Foreign participation is not the only difficult question NASA has to resolve, however. Although Congress has agreed to provide NASA with the \$150 million it wants in 1985 for preliminary design studies, both houses have signalled that they are not yet fully convinced of the need for the elaborate permanently manned facility planned by the space agency. Bills passed by the Senate and the House of Representatives instruct NASA to set aside several million dollars for "complementary" design studies of a less grandiose "man-tended" facility.

Some congressional champions of the space station objected to the requirement for the complementary studies, arguing that it would send "mixed signals" to the private companies looking at the possibility of investing in manufacturing and research in space. But most members felt it was prudent to keep options open as long as possible, in case budget retrenchment later in the decade should leave NASA with a half-completed "hotel in space".

Meanwhile, NASA is negotiating on a third front, with the US space science community. Although at the outset opposed to

the decision, space scientists have wasted no time in joining the planning effort to ensure that the scientific yield from the project is as great as possible. Earlier this month, a newly-established advisory committee on the scientific uses of the space station, under the chairmanship of Dr Peter Banks of Stanford University, recommended that the space station should be ac-

Commercialization of space

Whoever will pay the price?

Washington

US BUSINESS corporations are asking for extensive government subsidies and risk-sharing agreements before agreeing to take up President Reagan's call for the rapid commercialization of space. In Congress last week, leaders of three major aerospace corporations said that, without government help, the costs and risks associated with the exploitation of space are simply too high for it to be worth their while to be involved.

The witnesses, from the Fairchild, Grumman and McDonnell Douglas corporations, are all members of an industry-space commercialization committee which recently submitted a swathe of detailed recommendations to the White House. Although the report has not been published, it is clear that it calls for substantial tax incentives, some form of risk-sharing agreement and enhanced private sector access to the facilities and technical expertise of the National Aeronautics and Space Administration (NASA).

Mr Richard Kline, director of space station operations for the Grumman Aerospace Corporation, told the House of Representatives' subcommittee on space science and applications that the venture capital market rarely finances ventures costing more than \$75 million and is too small to support significant investments in space. To help companies reduce their initial investment risk, he said, NASA ought to provide "seed money" for the development of initial projects and reduce the flight of shuttle rides. He also called on NASA to expand its effort in promising areas of basic research such as microgravity and high vacuum environments.

Dr John Townsend, president of the Fairchild Space Company, said a number of complex fiscal and regulatory barriers would have to be removed before the private sector could exploit the full commercial potential of space. The major problem, he said, was that since space is not legally part of the United States, capital ventures based in space do not qualify for

company by a flotilla of compact free-flying modular platforms rather than the one or two large unpressurized platforms envisaged by NASA.

A minority of members of the committee is also anxious to persuade NASA to complement the space station, which is to maintain a 400-km east-west orbit at an inclination of 28.5 degrees, with a large free-flying platform in polar orbit. The polar module has been talked about within NASA but is regarded as a prime candidate for cutting in the event of budget difficulties or escalating costs. The platform, regarded as essential by the Earth-observation sciences, is expected to cost at least \$800 million.

Peter David

the 10 per cent investment tax credit. Congress, he said, should extend to all space ventures the special exemption it granted the commercial satellite industry in 1971.

The White House is expected to consider the private sector's shopping list in conjunction with a separate report on space commercialization drawn up by NASA itself but not yet published. Dr James Beggs, NASA's administrator, admitted that publication had been delayed because of internal disagreements but dodged questions about its specific recommendations. Meanwhile, he made it clear that all was not entirely well with NASA's first great commercial enterprise, its shuttle launch business.

Mr Beggs has two major worries. On the one hand, he is under pressure to reduce the price NASA charges for shuttle flights so that private companies trying to make a profit out of the United States' newly-privatized expendable launch vehicles (ELVs) will be better able to compete. On the other, he is afraid the Department of Defense (DoD), which was originally expected to account for a third of all flights in the 12-year life of the shuttle programme, may decide to divert a large proportion of those flights to its own ELV fleet. While officially describing its retention of ELVs as a "backup", DoD, which has yet to fly its first military payload aboard the shuttle, has been hinting that ELVs offer a cheaper and more efficient answer to its needs.

Only if DoD kept its military payloads for the shuttle, Beggs said, would the shuttle operation be able to recover its direct (but not its research, development and building) costs by 1988 or 1989. That was later than expected, because commercial flights began three years later than planned and the market for the shuttle turned out to be smaller than expected. Nevertheless, he said, NASA would start thinking, towards the end of 1986, about the possibility of selling or giving the shuttle fleet to private companies to manage at a profit.

Peter David

Karl Illmensee

NIH withdraws research grant

Washington

PROFESSOR Karl Illmensee, the embryologist at the University of Geneva accused of falsifying his nuclear manipulation experiments in mice, has now lost his research grant from the National Institutes of Health (NIH). The NIH action follows the finding by an international commission of inquiry in January that errors and discrepancies in Illmensee's records had thrown "grave doubt" upon the validity of the experiments. The commission was unable to substantiate or to dismiss the charges of deliberate fabrication.

Earlier this year, Illmensee's research grant from the Swiss National Fund was revoked. Illmensee has, however, resumed his duties at the University of Geneva.

Illmensee was supported by a \$218,000 NIH grant from May 1980 to April 1983. The grant had already been approved for renewal last year when the accusations of data falsification surfaced. On 18 May 1983, Illmensee, at the urging of colleagues who also obtained a now-disputed "confession" from him, wrote to NIH to withdraw a sentence in the grant application that he said had been included by mistake. NIH, concerned about the allegations of fraud and that the withdrawn sentence represented a "major element" of the research proposal, suspended the grant renewal.

After reviewing the commission's findings, NIH decided to terminate the grant application. The decision was taken by NIH director James Wyngaarden on 29 May of this year, but only made public last week after Illmensee had been informed. Wyngaarden also ordered NIH not to award further grants to Illmensee for the same work until he can validate the disputed results. In addition, the commission's findings are to be provided to any NIH review group considering a future grant application from Illmensee.

The NIH officials who studied the commission's report and other available evidence said they could not decide whether the withdrawn sentence in the application had contained "an element of invention", as the commission suggested was possible. But in recommending the actions that Wyngaarden approved, they said that because of "documented deficiencies", the grant should not be made. "In addition, the application was written two years ago and can no longer be considered an up-to-date, state-of-the-art proposal", the NIH officials concluded. The officials also said that no further investigation of the matter by NIH was necessary.

In the withdrawn sentence, Illmensee claimed that a chimaeric mouse had been produced by injecting nuclei from one cell line into mouse eggs of different parentage.

Illmensee at first said that the sentence was the result of a typographical mistake made in the haste to complete the application. After being pressed by the commission for an explanation of the actual origin of the chimaeric mouse, Illmensee attributed it to injection experiments involving another cell line; he also sent this revised explanation to NIH. But at the last meeting of the commission, in January, Illmensee said he was no longer certain of that explanation either.

The commission said "it is surprising that Professor Illmensee had to look through his protocols to discover which animal he was referring to in his original NIH application", and concluded that it was impossible to know which "if any" mouse was the chimaera referred to. Illmensee claims that the problem was one of two he has identified.

Neither the commission nor the NIH officials was able to draw any firm conclusions from the accusations of fabrication made by Illmensee's colleagues at Geneva.

Stephen Budiansky

Australian IVF

Orphan embryos

Canberra

LAWYERS in the State of Victoria are at sixes and sevens for lack of legal precedents for dealing with frozen embryos. The issue has arisen because Mrs Elsa Rios and her husband, the parents of two frozen embryos, died in an airplane crash last year, leaving a grown son and an estate estimated at more than \$1 million.

Using in vitro fertilization techniques involving donor sperm, an unsuccessful attempt was made by Professor Carl Wood and his colleagues at the Queen Victoria Medical Centre in Melbourne, in June 1981, to implant an embryo in Mrs Rios' womb. She then returned to the United States and the two remaining embryos have been kept frozen ever since. At present, under the common law, an embryo has no rights until it is born, and the State of Victoria will not enact a medically up-to-date legal prescription for questions of custody and inheritance from freeze-thaw embryos until the report of the Waller Committee on the whole subject appears in August.

The Right to Life Association has urged the state's attorney general, Mr Kennan, to appoint a legal guardian to protect the embryos so that they may develop to maturity. Meanwhile, in Los Angeles, twenty-five year old Mr Michael Rios, the son of Mr and Mrs Rios, is reported to have filed for guardianship.

Professor Wood says that the embryos are unlikely to survive because the techniques used at the time they were frozen were not as good as they are now, but no decision will be made about their disposal until after the Waller Committee reports and after correspondence with the executors of the Rios' estate.

Jeffrey Sellar

Childhood vaccine

US plans federal compensation

Washington

IF doctors, pharmaceutical companies and the families of the victims have their way, it will become the job of the US Government to compensate children who suffer damaging reactions to immunization. With the recent announcement by Wyeth Laboratories that it will no longer produce pertussis (whooping cough) vaccine because of the increasing liability burden, pressure has intensified for congressional action to assure that manufacturers will still be willing to produce the vaccines needed and that victims are adequately compensated. Pertussis, polio and measles-mumps-rubella vaccines are all now produced in the United States by single manufacturers.

Lawsuits against the companies are now common. About 60 of the 18 million children immunized each year with the combined diphtheria-pertussis-tetanus vaccine suffer serious long-term brain damage. The usual legal argument of the victims is that the manufacturer or the physician was negligent in failing to inform them of the risks of the vaccine. In some jurisdictions the manufacturer is responsible even when not demonstrably negligent. The companies have refused to discuss publicly the cost of settling these suits.

Under a legislative proposal drafted by the American Academy of Pediatrics and filed by Senator Paula Hawkins (Republican, Florida) and Representative Henry Waxman (Democrat, California), a compensation fund would be created out of surcharges applied to the vaccines. Victims would have the option of suing in the conventional way or of applying to the government for compensation through an administrative procedure. Both the pharmaceutical companies and the Reagan Administration would prefer a bill that prevents victims from suing.

Most of the childhood immunization vaccines used in the United States are purchased in bulk by the Centers for Disease Control (CDC) for distribution to state health authorities. The manufacturers portray their production of the vaccines as a public service, and claim they cannot raise prices to cover the burden of liability without engendering public disfavour. The compensation bill, which would cover all childhood immunizations, is in effect a legally-mandated price increase.

It has been suggested that behind many of the manufacturers' complaints about liability is a dislike of the low profitability of the vaccine business as compared with patentable drugs.

Vaccinations are mandatory before children enter school in the United States. CDC estimate that the pertussis immunization programme prevents 400 deaths per year.

Stephen Budiansky

Japanese legislative logjam

Educational reform held up

Tokyo

PRIME Minister Yasuhiro Nakasone has run into unexpectedly fierce opposition to his plans to set up a special commission to reform Japan's educational system. To push the necessary bill through the Diet, together with the bill for "privatizing" Japan's telecommunications monopoly (see *Nature* 308, 678 and 763; 1984) and some other controversial bills, Nakasone resorted in mid-May to an extension of the 150-day Diet session by a further 77 days. But opposition parties immediately responded with a boycott of Diet proceedings that lasted until mid-June. Debate has only just begun again, and it is still uncertain whether it will be possible to get the bills passed in the extended Diet session.

Moves to reform the educational system — particularly to prevent the university entrance examination hell — are certainly popular enough among the general public and Diet members. They jib only at Nakasone's insistence that it should contain only members appointed by him without the need for Diet approval ("to ensure neutrality"), that it should carry out its deliberations in private ("to avoid outside pressure") and should report directly to himself rather than to the Diet. Under these conditions, the major opposition party, the Japan Socialist Party, believes that the commission will simply be a rubber stamp for Nakasone's personal views on educational reform.

For the universities, Nakasone's ideas of reform are likely to centre on lessening the importance of the entrance examination. At present, university entrance is simply determined by total test scores in school-leaving examinations. Once entry has been won, very little further is expected of students — giving rise to the view of university as a "leisure land".

Nakasone's opinion, as spelled out in his book *A new theory of conservatism*, is that this should be reversed and that universities should be "easy to enter but difficult to graduate from". He also believes that the university system should be far more flexible, for example by allowing students to transfer credits from one university to another — a move that might well help to break down the overwhelming advantage that Tokyo University graduates have in finding jobs. The problem is that much of Nakasone's plea for more "choice" seems to rely on there being more private education, both at school and university levels; but private universities, according to their own just-released white paper, already see difficulties ahead.

With more than a third of all high-school students now going on to higher education, the number of undergraduates will increase massively between 1986 and 1992 when the second "baby boom generation" leaves school. But after that, university entrants

will decline sharply. The private universities point out that they need government support if they are to expand for a six-year period to take the extra students and then contract again. At present, the only way they would be able to cope with extra student numbers is by reducing the quality of education. Nakasone's reforms are thus becoming entangled with the level of state support — now declining — for private universities. As it is believed that Nakasone wants to appoint the present head of the private Universities Federation to leadership of the reform commission, the state universities believe that the whole reform plan might end up robbing them of funds.

The most vociferous opposition to the establishment of the commission has come, however, from the school-teachers' union *Nikkyoso*. The union claims a membership of 71 per cent of the primary and junior high-school teachers (though the Ministry of Education says the real figure is 51 per cent) and is left-wing in its views. At its annual convention last week — which required the presence of 2,500 riot police to prevent disruption by right-wing extremists as in the past two years — it decided to have nothing at all to do with the commission. Nakasone had been hoping that he could win support by having a *Nikkyoso* representative on the commission.

The union is naturally worried by the emphasis Nakasone places on "moral education". In March for example, he said in the Diet that "patriotism and the traditional Japanese respect for one's parents should be taught at school". To the left wing this seems too much like a return to the nationalist education that existed before the Second World War, and its members claim that signs of a swing to the right can be seen already.

The contents of school textbooks seem to be being more tightly controlled this year and the Ministry of Education has been accused of using its powers to dilute the facts of the darker side of Japanese history.

For Nakasone, much more rests on the successful passage of the bill than his own personal interest in educational reform. In November, he has to face the bi-annual elections for the Liberal Democratic Party leadership, success in which assures that he continues as prime minister. Despite his popularity abroad and his emergence as Japan's first international statesman, his performance at home has been lacklustre. He will be judged by his fellow party members mainly by his ability to handle the Diet, but in this session he has failed to get a single major bill through (although a measure to increase Diet members' salaries passed with great speed). As well as the educational and telecommunications bills, bills to reform the health system and the electoral system are waiting.

Alun Anderson

Tibetan geology

UK-Chinese joint venture

A COLLABORATIVE research project on the geology of the Tibetan Plateau has been announced by the Royal Society of London and the Chinese Academy of Sciences. It will run for two years from 1 July, with the actual fieldwork taking place in the summer of 1985. Fourteen Chinese scientists and twelve from the West (largely from the United Kingdom), under the leadership of Professors Chang Cheng-Fa, R.M. Shackleton and J.F. Dewey, will spend eight weeks covering more than 1,000 km in perhaps the most inaccessible part of the world. The estimated cost of approximately £350,000 is being shared roughly equally between the two academies, with the Chinese providing the back-up for the expedition.

Scientifically, the project is complementary to the joint French-Chinese investigations in southern Tibet in 1980–83, as a result of which earth scientists now have a better understanding of the mechanisms of formation of the Himalayas and other linear mountain belts. In contrast, the means by which vast areas of relatively undeformed rocks can be uplifted (to an average elevation of 5 km in the case of the Tibetan Plateau) are still obscure, in spite of theories based largely on consideration of the rheology of the Earth's crust. The Anglo-Chinese project should provide the hard facts against which these theories can be tested.

The project involves a 1,000-km traverse from Lhasa to Golmud, on the northern edge of the Tibetan Plateau. Geologically, Lhasa sits on a block representing the southernmost part of Asia, at the junction with the Indian block to the south. North of the Lhasa block, the geology is poorly known, although a number of crustal units have been recognized. Only painstaking fieldwork, building on the reconnaissance surveys of the host nation, will clarify the relationship between these units.

The major results of the French-Chinese work relate to the past 20 million years, although reports of the second expedition in 1981 (see *Nature* 307, 17–36; 1984) suggested that Tibet may consist of pieces broken off Gondwanaland, which moved north in advance of India, perhaps in successive waves, and eventually collided with Eurasia. The British aim to delve deeper into the past, using predominantly palaeomagnetic and faunal studies, to the earliest separation 150 million years ago in the late Jurassic. The detailed histories of these continental fragments may be better known as a result of the new project.

Negotiations started in 1981 and the programme is regarded as a landmark in the continuing expansion of collaborative programmes between the Royal Society and the Chinese Academy. **Peter Gambles**

Dutch nuclear issues

Decisions later

Waalre, The Netherlands

THE Dutch preoccupation with nuclear energy, military and peaceful, will continue for at least another eighteen months. That is the effect of the decision of the Dutch Parliament on 13 June that a final decision on the siting of US cruise missiles in the Netherlands will be made only at the end of 1985. The Netherlands is the only country out of step with the policy of the North Atlantic Treaty Organization (NATO) in this respect.

Never has a subject been discussed so feverishly in the Netherlands. Only the political parties on the right (conservatives and four smaller parties) are in favour of deployment of the missiles, but, according to opinion polls, voters are against the siting plan. On the left, the socialists and three small parties are against, as are the great majority of their voters. The role of the Christian democrats, which together with the conservatives form the government, is especially confusing. Their voters are sharply divided, and they are under heavy pressure from the churches which in advance of 13 June issued declarations asking the government not to install the missiles and in the process offended some church people who hold that the declarations were "too political".

Similarly, the development of civil nuclear power in the Netherlands is on ice, with more than half of the population against it. There are two small nuclear plants (of 60 and 470 MW) now operating, and ten years ago the government proposed building three 1,000-MW plants. But that plan was shelved after a year and a half because of the strength of the opposition, unabated by the oil crisis. But discussion continues, with the churches and the environmental movement playing a prominent part.

Earlier this year (see *Nature* 307, 405; 1984), it seemed the issue had been settled by the report of the officially organized public consultation which in the previous two years had gathered evidence for its conclusion that further nuclear plants are now necessary and that combined heat and power schemes, in industry and domestic applications as well as wind power, should replace obsolete plants in the 1990s. But now it seems that the government is brooding on plans to expand nuclear capacity by 4,000 MW, and leading newspapers have been complaining that this overrides the democratic consultation the government itself had organized.

The government's plans for nuclear power will in fact be published in September but meanwhile the minister of economic affairs, Gijs Van Aardenne, who was unenthusiastic about the report on nuclear power published in January, does not conceal his enthusiasm for further development, while Prime Minister Ruud

Lubbers, in 1974 the minister of economic affairs who put forward the earlier nuclear power plan, is also a supporter.

So the months ahead will be another test for democracy in the Netherlands. Although the debate about the missiles will continue for 18 months, it is barely conceivable that the Netherlands will not follow its NATO partners, despite five years of public demonstrations and social unrest. And the development of nuclear power may follow the same route.

Casper Schuurin

UK science budget

Money needed

THE British Government's advisers on the share-out of the civil science budget have warned Members of Parliament of the dire consequences for the nation's research enterprise unless greater provision is made for science over the next decade.

The House of Commons Select Committee on Education, Science and the Arts, which has started an inquiry into the science budget, heard last week from the Advisory Board for the Research Councils (ABRC), represented by its chairman, Sir David Philips, Dr Walter Bodmer, director of research of the Imperial Cancer Research Fund, and Dr Derek Roberts of General Electric Company Ltd. On the basis of published government expenditure estimates, ABRC predicts that the real value of scientific research is set to decrease by 25 per cent over the next decade.

This stark prediction arises from a number of effects that diminish the value of the research budget, which at first sight is planned to remain roughly constant in real terms. Firstly, according to ABRC, the science vote has had largely to compensate for an 11 per cent cut in recent years to university support of science from the University Grants Committee. Secondly, the Treasury's assumed annual cash increase for the science vote, which decreases from 3 per cent per year to 2.5 per cent per year over the next decade, is fully 2 per cent less than the Treasury's estimated annual increase in the retail price index. This alone would produce a decrease of 17 per cent over the 10 years in real terms.

On top of this, there is the problem of superannuation payments for the increasing number of retired research council staff. And the perennial problem of subscriptions to international organizations still remains. ABRC members emphasized last week their conviction that science had lost out in recent years in relation to other publicly supported activities, while the increasing sophistication of scientific equipment has meant that the same amount of money bought less. And they were disturbed that the government's published expenditure plans include no special reference to the value of science in the economy.

Tim Beardsley

Acid rain

UK in minority of one

THE British reputation for waywardness in the eyes of many Europeans seems unlikely to be improved this week at the World Conference on the Environment, taking place in Munich at the behest of the government of West Germany to discuss ways of combating atmospheric pollution. At the opening session on Monday, Dr Martin Holdgate, chief scientist of the British Department of the Environment, declined to give any firm commitment to reduce British emissions of sulphur dioxide, saying only that a range of measures was being considered that would allow Britain to reduce emissions "in a cost-effective way".

In Germany, acute concern over damage to forests is leading to pressure for drastic reductions in acidic emissions, chiefly sulphur dioxide from power stations and nitrogen oxides. But Dr Derek Pooley, chief scientist at Britain's Department of Energy, said last week he was "convinced that sulphur dioxide was not the major culprit in causing damage to forests".

A major study on acid deposition published last week by the department's energy technology support unit emphasizes the uncertainties. Forest damage appears not to correlate well with sulphur dioxide concentration when dry deposition from local sources is excluded. But above an altitude of 600 metres, which is where most of the damage occurs, photochemically produced ozone can reach sustained and high concentrations. Although no mechanism for ozone damage is known, one suggestion made in the Department of Energy's study is that ozone causes damage to leaf cuticle, which in turn allows leaching of magnesium as the pH falls. But Dr Bill Binns of the Forestry Commission, who has made

Cost of impure mice

Washington

A UNIVERSITY of Wisconsin researcher has won an out-of-court settlement with Charles River Breeding Laboratories over a mix-up of mice that the researcher said caused her to waste a year's work and set back her career. Dr Brenda Kahan will receive an undisclosed monetary settlement; in addition, the University of Wisconsin will receive \$40,000 from Charles River as compensation for research and overhead expenses of the invalidated research. Two-thirds of that \$40,000 will support Kahan's research.

Charles River, a major supplier of laboratory mice, shipped large numbers of genetically impure mice to researchers who believed they were getting a pure strain. The company says it has now corrected the problem.

Stephen Budiansky

several studies of forest damage in West Germany, is not so quick to rule out a role for sulphur dioxide. He emphasizes that "occult" precipitation — fog and mist — can be much more acidic than rain and may yet play a part, perhaps in conjunction with other stress factors.

If there is still mystery over the mechanism of damage to trees, the mechanism of damage to fish in fresh water is rather better understood. It seems that elevated levels of inorganic aluminium may be a major cause of mortality. But the chemical changes that will occur in a body of water subjected to acid deposition within its catchment area are greatly affected by the nature of the soil and bedrock, as well as by the type of vegetation present. It is clear that coniferous trees, in particular, can efficiently filter out acids from the air and release these in solution at the next rainfall. The Forestry Commission in Britain is already investigating management practices that may reduce acid release into streams.

Because of the multitude of factors that affect acid depositions, the precise attribution of blame is difficult or impossible. Motor vehicles, for example, along with power stations may be (indirectly) a major source of ozone, through their emissions of nitrogen oxides. But the British contingent at Munich this week will doubtless be able to make much of new estimates published by the Department of Energy of the proportion of British-emitted sulphur dioxide that finds its way to other countries: 3.6 per cent to West Germany, 4.4 per cent to Norway and Sweden combined, while almost 30 per cent stays in Britain.

While the British Department of Energy harks on the quantitative uncertainties of acid deposition, the Nature Conservancy Council, which also last week published its views on the matter, takes a somewhat different line. It argues that acidification reduces species diversity in aquatic systems and that the only long-term solution to the problem is therefore to reduce emissions of sulphur and nitrogen oxides. And it is opposed to the policy of liming affected waters on the grounds that the practice will in the long term bring its own undesirable effects.

The British Government seems likely to find itself increasingly isolated over acid emissions. The government is committed to reply before the end of the current parliamentary session to the latest report of the Royal Commission on Environmental Pollution, which urged that the Central Electricity Generating Board should introduce emission abatement measures on a pilot basis. And the Select Committee on the Environment of the House of Commons is shortly to report on the results of its inquiry into "acid rain", which, it may be guessed, will not take a complacent view. It seems likely that political pressure will before long force some government concessions.

Tim Beardsley

Pesticides

New chemicals under fire

THE latest returns in a two-year survey of the British barn owl population suggest an even greater population decline than had been feared. Ten years ago there were some 4,500–9,000 pairs in England, Scotland and Wales and now, after 18 months of the "Barn Owl Survey" organized by the Hawk Trust, the indications are that the overall decline has been at least 10 per cent, with much greater reductions in many areas.

The only areas where there has been little decline are in Scotland, where the Forestry Commission's activities have provided some additional refuge for the dwindling population. The population estimates, however, are becoming increasingly inexact as the numbers involved get smaller.

The continued decline of the barn owl is thought, says Colin Shawyer of the Hawk Trust, to be a result of the use of new rodenticides by British farmers. Difenacoum and brodifacoum have been growing in popularity in the past few years, steadily replacing warfarin, which is known to be relatively non-toxic to species other than rodents. Brodifacoum is thought to be especially toxic to birds and for that reason has not been given full approval by the Ministry of Agriculture, Fisheries and Food under the Pesticides Safety Precautions Scheme.

Some of the evidence for the effects of the new pesticides comes from Malaysia. On some oil palm plantations, the combination of warfarin and the wild barn owls, encouraged by the provision of nestboxes on poles throughout the plantations, has effectively controlled severe rat infestations. But even on estates where there has been no evidence of warfarin resistance, the new pesticides have been tried. And, says the Hawk Trust, barn owl numbers declined so rapidly that the estate managers went back to the old methods.

In Britain, some farmers have reported

to the Hawk Trust that barn owls seemed to disappear from their farms soon after they started using the "second generation" rodent baits. And a veterinary surgeon reported that a barn owl thought to be dying from the effects of difenacoum poisoning was successfully treated by the vitamin K antidote to the pesticide.

Much of the evidence on pesticide toxicity is circumstantial, the Hawk Trust concedes. So the urgent need is for more research on the effects of the new chemicals on non-target species.

Charles Wenz

Irreproducing ibis

Tokyo

EFFORTS to save Japan's *Nipponia Nippon* (the Japanese crested ibis or "toki") seem doomed to failure after a romance between two of the remaining birds — Kin (the female) and Midori (the male) — ended in a mid-mating season fight. As Kin is now, in human terms, around 80 years old, the chances of a successful mating next year are thought to be close to nil, although hormones are to be administered in the hope of keeping her in breeding condition.

The toki, a large white bird with a characteristic long downwardly curved black bill and a red face, was a common sight in wet paddy fields and ponds throughout Japan and parts of east Asia as recently as one hundred years ago. But a catastrophic decline in their numbers this century led to their being given special status in Japan in 1934. The war prevented any serious conservation measures, however, and by 1953 only 31 birds remained.

In 1966, special measures were begun but numbers continued to decline so, in 1981, the remaining five wild birds and one captive bird were removed to a breeding centre on the island of Sado — where in bygone days troublesome cadet branches of the imperial family were exiled so that their lines might disappear. Two birds died but hopes rose in 1983 when two of the remaining four birds paired. The female died, however, from a blocked fallopian tube shortly before she laid her eggs.

This year, the romance between Kin and Midori was followed closely but to no avail. As well as being in ripe old age, Kin, having been reared in captivity, apparently did not take too well to Midori, who had led a life in the wild until 1981.

The Sado Island Toki Centre is now to advance research on artificial insemination as fast as possible, but researchers doubt whether practical methods can be developed in time. Hopes for saving *Nipponia Nippon* from extinction now rest largely with the Chinese. In 1980, seven birds were found in China, raising the known numbers there to fourteen — the same as the number remaining in Japan. There have been occasional rumours of sightings in Korea and the southeastern Soviet Union, but none has been confirmed.

Alun Anderson

IMAGE
UNAVAILABLE FOR
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REASONS

Victims both — a barn owl and prey.

Esca K. Thompson

Science and research in India

SIR — You say in your study of science in India that there is no dearth of funds. That is true. Organizations such as ICMR, CSIR, DST and UGC have enormous funds. But how much of this money goes to deserving people? Who, in any case, is deserving? In my opinion, those who deserve research funds are those who have worked in a specific area. Yet as things are, persons who have never seen tribes, never travelled in forest areas and have not worked on any aspect of anthropometry, haemoglobinopathies or population genetics may be allowed to spend 5 million rupees at the expense of investigators with relevant experience.

Last year, I asked a senior official of a major funding agency about this practice. His chilling answer was that "we do not wish even to look at projects costing less than three lacs" (300,000 rupees). He further added that nothing can be done in science these days with one or two lacs of rupees. The truth is that there appears to be no remedy.

In India, there are few first-rate academic institutions when compared with the enormous number of students graduating every year. The general decline of students' interest in higher studies and the mushroom growth of colleges everywhere are unrecognized impediments to India's progress. The tolerance and even the cultivation of corrupt practices in the name of democracy is rampant in all universities and colleges, and in the publication of research work, as discussed in your report.

In my view, four out of five of our universities do not have enough money to support the basic necessities of modern science education, despite modest support from the University Grants Commission. The first reason is the apathy of state governments towards higher education, the second, the outlook of the bureaucrats who control events and, the third, the incompetence of the vice-chancellors. Nowhere in the world have vice-chancellors such an uncertain future (they are replaced so often) and so little power. Even those with vision and ability can be quickly cowed by an under-secretary of the state government on any trivial matter. The exchange and extraction of mutual benefits is rife in the entire university education system. So I would modify *Nature's* slogan "Excellence in the midst of poverty" to "Excellence against all odds".

My twenty years of teaching experience lead me to conclude that most talent is distracted from research. Many faculty positions, research scholarships and senior fellowships are lying vacant and at least 60 per cent of research scholars leave research fellowships before completion. Nobody wants to acknowledge that part of the reason is the victory of incompetence over ability, mediocrity over genius. In my view,

corrupt politicians and officials poison social progress only slowly, but corrupt teachers and particularly scientists can spoil generations. No nation should aim at nurturing mediocrity, whatever its national beliefs.

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SIR — In my experience Indian scientists have the zeal and energy to succeed, but are handicapped by limited facilities and experience. The majority of institutes in India are sub-standard and producing graduates with limited practical knowledge, as boldly accepted by Dr Subramanian (*Nature* 307, 206; 1984). One reason for this is that institutes away from the centre suffer step-mother treatment. The centre and the University Grants Commission, the two main funding authorities, are too easily influenced by individuals heading the institutes governed by the centre, or by centrally located organizations who can approach the funding authority directly.

To obtain a research grant in India, one has to be not scientifically orientated, but politically motivated. The system brings non-scientifically minded individuals to the top who are against the return of their scientific compatriots from abroad, and cannot see their local colleagues rising quickly unless they share the credit for their work. Dr Balasubramanian (*Nature* 307, 321; 1984), sitting in one of the central institutes, has perhaps never had a chance to see establishments which lack the basic necessities to teach or initiate scientific research.

The main problem facing the plan to site an international centre in India (*Nature* 305, 350; 1983) is that of supply of basic chemicals. Local products are not up to standard, and to import foreign goods, even after going through the red tape, one may have to pay 110 per cent customs duty on essential consumables of research. Sometimes it takes days to get supplies through customs.

Admittedly, India has its nuclear and space programmes to show the world but I do not agree with the claims of Dr Balasubramanian, who is perhaps unaware of the high prevalence of leprosy, tuberculosis and other communicable diseases in the very state where he is working. India must concentrate on educating society and research programmes should aim directly to reduce such human suffering. Many expatriate Indian scientists may wish to go back and the idea of a technology city is very rosy to them, but unless this city is controlled by a scientist with special powers to cut through the bureaucracy of Indian authorities, the city will be a failure. To

raise the existing standards of Indian research, direct communication and collaboration with international scientists should be promoted.

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SIR — In your otherwise interesting survey, you were not able to give much time to the decaying but historical city of Calcutta, the first capital of British India, and the cradle of modern science in India. You rightly mentioned the late Sir J.C. Bose, the first Indian physicist FRS (1920) and his Bose Institute, founded in 1917 on the model of the Royal Institution of Great Britain. You also mentioned the late Professor S.N. Bose, FRS (of Bose-Einstein statistics) and his institute. But you omitted to mention a giant of Indian science, the late Professor M.N. Saha, FRS, a class-mate of the late S.N. Bose. It was after their joint English translation of Einstein's relativity papers in book form (published by Calcutta University) that Professor Bose was able to continue his work on his chosen subject while at Dacca University.

Professor Saha (the third Indian physicist FRS elected in 1927) made his impact on the world of astrophysics through his famous thermal ionization work in 1920, and became the first Khaira Professor of Physics, Calcutta University in 1921. After a spell at Allahabad University on his return to Calcutta in 1938 Professor Saha installed the first Indian cyclotron at what is now the Saha Institute of Nuclear Physics, inaugurated by the late Madame Joliot-Curie in 1951 with Saha as the founder-director. This institute also built the first electron microscope in India, and now has a thriving biophysics division.

In the premises of the Calcutta University Science College another great Indian scientist, the late Professor S.K. Mitra, FRS, established the Institute of Radio Physics and Electronics.

Third, at the Presidency College, Calcutta, the late Professor P.C. Mahalanobis built the nucleus of the Indian Statistical Institute. It was at Presidency College that the first cloud chamber for cosmic ray studies was constructed by Professor Rajen Sengupta, then a student of the late P.M.S. Blackett.

Finally, at Calcutta today the most active centre for scientific research is the Centre of Advanced Study on Cell and Chromosome Research, directed by Professor A.K. Sharma (in collaboration with his wife Mrs Archana Sharma), at the Department of Botany, Calcutta University. Professor Sharma's new technique for the study of the physical and chemical nature of chromosomes has been widely adopted all over the world.

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Lights hidden under bushels

Geochemists are not alone in failing to make clear the elementary assumptions on which they work. The result is that what they do is under-appreciated.

GEOCHEMISTS, who have a great many achievements to boast of in the past few years, are sometimes their own worst enemies. That is what many innocent readers will conclude from a casual inspection of the rush of articles with which the geophysical literature is now spattered, and which purport to show how, for example, the measurement of neodymium isotopes in some sample of rock can shed light on the past history of the material. The articles on pages 753 and 773, singled out perhaps unfairly as representative of their kind by the mere accident that they are included in this issue, show how unforgiving of their readers' patience the geochemists have become. (If they should protest that they are not alone among specialists in making the assumption that everybody who matters knows what they intend to say, they are merely making the more dangerous assumption that only fellow specialists are likely to be interested).

The underlying difficulty is common in new fields of investigation, perhaps especially in those almost certain to become important. It is then tempting for those with new things to say to work on the principle that the assumptions with which they live day by day are widely appreciated. If indeed, with the passage of time, the novel branch of geochemistry based on the analysis of terrestrial (and other) rocks for the radioactive decay products of rare but long-lived components of the Solar System turns out to be as important as it promises, the result will be that the general readership of the literature will know what the assumptions are. This, to a large extent, is what has happened in the previously daunting field of nucleic acid chemistry, where it is no longer necessary for people to spell out the simple fact that DNA molecules are constructed from four distinct subunits, for example. But if the new geochemistry should be only of middling importance, is it destined to remain more or less a closed book?

That would be, to say the least of it, a pity, which is why, at least for a little while, geochemists should be a little less austere in the way they describe what they have done. It is not that the field is all that unfamiliar. Indeed, it resembles in many ways the pioneering work of F.W. Soddy, in the first decade of this century, in seeking to determine the age of the Earth from the radioactive decay of uranium and thorium.

The essence of that task, successfully

completed in the 1930s, is that two principal isotopes of uranium (with mass numbers 238 and 235) ultimately yield as decay products two isotopes of lead (with mass numbers of 206 and 207 respectively). Thorium-232, on the other hand, finishes up as lead-208.

What this implies is that it is possible to infer the length of time for which a rock containing uranium has been undisturbed by a sufficiently accurate measurement of the amount of, say, uranium-238 and of the abundance of lead-206 relative to that of some other isotope of lead known not to be the product of radioactive decay. (Lead-204 is the common standard.) For then the lead-206 found in a sample of the rock will represent both that formed by the decay of uranium-238 and that present at the outset. If the *primaevae* abundance of lead-206 relative to lead-204 is known (as, for example, from the analysis of rocks containing lead but no uranium), it requires merely simple subtraction to discover how much lead-206 has been formed by the decay of uranium-238.

The techniques now being exploited with such profit by the geochemists are in principle no different. Neodymium-143 is formed by the alpha-decay of samarium-147 (with a half-life of more than 100,000 million years) and strontium-87 from rubidium-87 by beta-decay (with a half-life of nearly 50,000 million years). Relative to other isotopes of neodymium and strontium, the excess amounts of the radiogenic daughter elements in some sample of rock are simply related to the amounts of the radioactive isotopes originally present and to the length of time which has elapsed. The conventional standards for comparison are the isotopes neodymium-144 and strontium-86 respectively.

Much of the confusion that attends the use of these techniques in geophysics stems from the recognition, in itself a discovery of great importance, that the elements involved, the radioactive parents and their daughters, have been repartitioned among the several components of the Earth's layer structure during geological history, not all of it Archaean. Both rubidium and strontium, as chemical elements, are more abundant in continental crust than in the mantle from which the continents were formed, but the relative concentration of rubidium is greater than that of strontium (by factors of perhaps 70 and 20 respectively). With

samarium and neodymium, by contrast, the daughter tends to be more highly concentrated than the parent.

Chemists are quick to point out that this redistribution of material is largely a function of the radii of the ions of the elements involved. (By chance, uranium and lead are concentrated in the continental crust relative to the mantle by roughly the same degree.) Whatever the mechanism of this process, what emerges is that the abundance of strontium-87 (relative to strontium-86) is greater in the continental crust than in the mantle, while the abundance of neodymium-143 (relative to neodymium-144) is less than in the mantle.

The interpretation of the ratios actually measured is complicated by the difficulty of knowing the full history of the material concerned. In attempts to infer the age of the Earth from measured abundances of uranium isotopes and the corresponding isotopes of lead, it is sufficient to search for the oldest rocks and to compare the results of abundance measurements with those obtained from meteorites. By contrast, the chief utility of the use of the newly-measurable isotopes is that they may be used to trace the comparatively recent history of the Earth's surface layers, continental crust and ocean floor alike, in which case there can be no fixed assumptions about the abundance of the isotopes in the starting material.

This does not, however, prevent practitioners from talking of "anomalies". The reference point is usually some notional composition of *primaevae* material but is hardly crucial, since only differences of abundance bear on the processes by which material has most recently been repartitioned. Eventually it should also be possible to construct a kind of historical record of the average abundance in the mantle of the isotopes involved, at least if the mantle is crudely homogeneous.

All this, of course, is commonplace among geochemists. But now and again, it would do no harm if they were to remind their readers that neodymium-143 is the product of the radioactive decay of samarium-147, or that lead-206 is the product of uranium-238. Certainly they should be more forgiving of their readers' almost certain ignorance of the importance of these isotopes as tracers of the fractionation of terrestrial material. To fail to make these concessions is to underplay an exciting field.

John Maddox

Fisheries

Genetics, dynamics and politics

from John H. Lawton and Robert M. May

MARINE fish stocks, broadly interpreted to include such taxonomically disparate beasts as whales, shrimp, crabs, shellfish and squid, together with fish in a strictly zoological sense, constitute one of the world's great biological resources. Attempts to understand, conserve and exploit resources confront marine biologists with a number of intriguing theoretical and practical problems, often made more difficult by political and economic constraints operating on the fishery. A recent Dahlem Conference* grappled with a range of such problems.

Some of the biological problems confronting fisheries managers have implications for evolutionary biology and ecology far outside their primary concerns of maximizing profits and minimizing the risks of overfishing. Two, namely fishing as a major selective force acting on the genetic composition of fish stocks and the problem of how best to construct tractable models of complex multi-species fisheries, seem particularly important.

Often, though by no means always, fishing a previously unexploited stock leads to faster growth and earlier breeding of the surviving population¹. Predictions of yield, profits and the population's capacity to withstand exploitation hinge on a sound understanding of such changes in demography under exploitation. The changes can happen quickly, and have generally be attributed to the fact that as stocks are fished down, food supplies per individual increase, leading to faster growth and earlier maturity. However, an explanation based on such density-dependent effects in the population dynamics is not the only possibility; the response could also be genetic; if, as seems likely, growth rates and age of maturity are genetically determined, and there is heritable variation for these traits within the population, then fishing represents a massive novel selection on the population. Individuals that grow faster and/or mature earlier may be at an advantage in the fished population because, other things being equal, they are more likely to reproduce before being caught. Other mechanisms possibly responsible for the observed demographic changes include alteration in environmental variables that more or less accidentally coincide with changes in fishing intensity, or even that apparent changes may be artefacts of the way the data are gathered.

Although it is difficult to distinguish genetic from other explanations for changes in the vital rates of exploited fish

stocks, useful tests may come from situations where fishing, paradoxically, leads to a reduction in growth rates and to an increase in the mean age of fish. This apparently happened in the case of the Lesser Slave Lake Whitefish, *Coregonus clupeaformis*²; such changes are not easily accounted for by responses in population dynamics to fishing, but may be due to heavy selection of gill nets on large rapidly-growing genetic variants in a species where reproduction is strictly determined by age and not by size².



In most populations we might expect to see both population-dynamic and genetic responses to fishing, working either in concert or in opposition to one another to influence the demography of the exploited population. The consequences are important for managers wishing to predict the long-term dynamics of fish. But over and above all these practical considerations, evolutionary biologists, interested in life-history strategies³, should pay more attention to the possibility that fish life-histories can evolve in response to fishing.

A second area where essentially practical problems of fisheries management may have wide theoretical repercussions centres on how best to construct tractable models of complex multi-species communities. Not unexpectedly, the fisheries that are most rich in species are in tropical waters. For example, off the Senegal-Mauritanian coast as many as 174 species are involved in the demersal fishery; a single trawl haul can contain 20–50 species. Official statistics for this area tend to group catches into commercial categories, but still 48 species are identified in the records for Senegal⁴. Classical fisheries models, based on single species, are obviously no use under these circumstances. However, detailed simulation models involving all the commercial

species and their food supplies are not a solution either. Even if the data were available, the sensitivity of the very complex models to errors inherent in estimating the snowstorm of parameters necessary for their construction makes them unworkable.

Simplified models are much less sensitive to parameter variation, but the problem is how to simplify them. Grouping species into manageable sets could be done in several ways, for example taxonomically, or by habitat, or by commercial value. At the moment, there are no reliable theoretical rules to guide such endeavours, and the best solution must, in any case, depend on the circumstances and the objectives of the modeller. One promising approach may be to use some combination of body size and habitat. Even the very complex West African fishery can be grouped into a limited number of species assemblages on the basis of habitat, specifically water depth and bottom type⁴. Within habitat divisions, grouping individuals by body size irrespective of taxonomy may not seem the most obvious thing to do, but since body size has rather predictable effects on an animal's vital rates⁵, the approach has much to commend it. In the long term, successful predictions using aggregated models of complex multi-species fisheries could have profound implications for ecologists struggling to make sense of, and model, equally complex communities in other habitats.

Of course, even classical 'single-species' fisheries, such as cod or herring in the North Sea, or anchovies of Peru, are members of complex communities, providing both food and shelter and harbouring enemies and competitors⁶. A broader 'ecosystem' perspective on even a simple single-species fishery may suggest ways of using indicator species — sea-birds, for example — to monitor the health of fish stocks. There was disagreement among participants at the Dahlem Conference about the feasibility of such an approach, but it should not be dismissed out of hand. Certainly, reduced breeding success, or failure of sea-bird colonies to recover from a natural disaster that in the past has presented no problems, could be a clear indication that all is not well with the birds' food supply.

Another major theme of the meeting was the way unpredictable environmental events can lead to significant fluctuations in fish stocks, and thus to uncertainty in the catch from year to year, or over longer periods. In particular, it can be that the rates at which new recruits are produced are affected differently by some kinds of environmental fluctuations when the stock density is relatively low (as a result of heavy exploitation) than when it is relatively high (in its pristine state); this, in turn, may result in the yield becoming significantly more variable as fishing pressures increase. As observed at Dahlem^{1,4} and before⁷⁻⁹ such effects can lead to situations where it is better to fish less intensely, diminishing the fishing effort below that estimated to

* The Dahlem Conference on 'Exploitation of marine communities' was held in Berlin on 1–6 April 1984. The proceedings will be published as a book by Springer-Verlag, later this year.

give the maximum average yield in order substantially to reduce the severity of fluctuations in the yield. Such trading of risk against maximum possible return is more familiar in the management of university endowments.

In this general context, the conferees sought to identify some tentative patterns in the degree to which different kinds of fish stocks exhibit such variability. One suggestion was that species high in the food web tend to live longer, which in turn tends to make their population densities relatively less variable¹⁰. Another suggestion was that tropical species tend to live longer than ecologically similar temperate ones, again tending to make for relatively steadier dynamics¹⁰, although for terrestrial insects Wolda and others have concluded that tropical populations do not fluctuate significantly less than temperate ones^{11,12}. A more specific suggestion, tied to life-history details, is that species such as plaice or sole, which make their living on the essentially two-dimensional world of the sea floor, are thereby in a less chancy environment, and that this is why their stocks appear to fluctuate less than do many pelagic fish such as herring¹³.

Troade (Institut des Pêches Maritimes, Nantes) and others emphasized that fluctuations in the processing and marketing of fish are every bit as worrisome as fluctuations in the biological stock or in the catch. For fisheries that are relatively predictable, it may be desirable to bring industrial capacities for treatment, packaging and marketing fish products close to the minimum required to exploit the resource at some set average level, allowing for some fluctuations but not for gross overexpansion in good times and collapse in bad ones. This is easier said than done, but in the absence of such efforts at control then all factors — from biological harvesting to marketing — can create lags and inertia that end up greatly amplifying such fluctuations as are inherent in the system. Conversely, for fisheries that are intrinsically highly variable (either in yield or in species composition), as some pelagic fish seem to

be, it may be best to aim for flexibility in harvesting, processing and marketing, given that 'cashing in' on the good years will have little repercussion on future biological events. Tidy though such a dichotomy is in principle, we really need to understand multispecies effects better before we can implement the ideas with any confidence¹⁰.

As emphasized by Clark (University of British Columbia) and others in discussion, the real uncertainties in fish catches and marketing are further complicated by psychological aspects of uncertainty. Thus, for example, the macho acceptance of risks by some kind of fishermen can confound policies based on conservative rationality. Despite all the biological, economic, political and psychological problems that beset the management of both

single-species and multispecies fisheries, the general tone of the final discussion was, we think, a cheerful one: our current understanding, though very far from complete, is probably enough to provide a basis for giving good advice for the management of most fisheries, at least in the short run. But in the long run, as stressed by Shepherd¹⁴, in most cases we probably need kinds of information (knowledge of the absolute magnitude of stocks, and a full understanding of factors determining recruitment and mortality) that is not currently available. □

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Materials science

Superlattices, superdislocations and superalloys

from Robert W. Cahn

SUPERALLOYS, the key materials for making turbine blades and discs in jet engines, have been steadily improved since the introduction of the wartime 'Nimonic's; maximum service temperature has been progressively raised and engine efficiencies have been improved in parallel. But the available means for maintaining this rate of improvement are now more or less exhausted short of a radically new approach. Just such an approach is offered by some recent 'pure' research on one constituent phase of all superalloys, the nickel-based 'gamma prime' phase.

The diverse family of superalloys is, in essence, based on a single structural principle. They consist of a dispersion of particles of a coherent 'gamma prime' phase — Ni₃Al in its pure form but generally containing Ti and other elements — within a gamma phase, a matrix of nickel containing various solutes. Gamma and gamma prime are coherent in the sense that the lattices match up so closely that their interface is of very low energy; this in turn implies that the gamma prime particles are highly stable against growth. The other vital characteristic of these alloys is that the gamma prime phase is atomically ordered; in other words, it consists of a superlattice, like the much-studied Cu₃Au alloy, but unlike that alloy the order persists undiminished right up to the melting temperature. All sorts of other microstructural features have been introduced to add to the excellent creep resistance of superalloys at high temperatures, but the above features are the crucial ones.

Gamma prime has a remarkable property: whereas all ordinary alloys weaken as they are heated, the strength of an experimental alloy of pure Ni₃Al gamma

prime phase increases as it is heated, almost until it melts. Plainly, this property is of central importance in determining the good creep resistance of superalloys as a class. Such anomalous mechanical behaviour as Ni₃Al and a number of other A₃B phases, such as Ni₃Ti. Much ink has been spilt in attempts to interpret this anomaly. It is known that dislocations move through highly ordered alloys in pairs, collectively termed 'superdislocations'. In a very stably ordered alloy these pairs of dislocations, which are joined by strips of disordered material called antiphase domain boundaries, are so close together that they can twist and turn and escape on to abnormal lattice planes. It is the twisting and turning that leads to the anomalous mechanical behaviour; at least, this is the current orthodoxy.

In superalloys cast in the normal way, the individual gamma prime particles — typically less than 1 µm across — each consist of a single domain. Consequently, it has not been possible to manipulate their domain size, which is an important variable in the general theory of the mechanical behaviour of ordered alloys. This situation has recently been changed, however, by research carried out in the Japanese laboratory at Tohoku University, where so much important metallurgical research has been done. An important paper from this group examines the behaviour of superalloys ultrarapidly quenched from the melt (Inoue, A., Tomioka, H. & Masumoto, T. *Met. Trans.* 14A, 1367; 1983). At cooling rates approaching 10⁶ K s⁻¹, there is not time for proper ordering and the phase consists of fine antiphase domains around 50 nm in size. The result, for a variety of phases of type Ni-Al-X, where X is Cr, Mo

1. Sissenwine, M.P. in *Exploitation of Marine Communities* (ed. May, R.M.) (Dahlem Konferenzen, Berlin, 1984).
2. Handford, P., Bell, G. & Reimchen, T. *J. Fish. Res. Board Can.* 34, 954 (1977).
3. Istock, C.A. in *A New Ecology. Novel Approaches to Interactive Systems* (eds Price, P.W. et al.) (Wiley, New York, 1984).
4. Gulland, J.A. & Garcia, S. in *Exploitation of Marine Communities* (ed. May, R.M.) (Dahlem Konferenzen, Berlin, 1984).
5. Peters, R.H. *The Ecological Implications of Body Size* (Cambridge University Press, 1983).
6. Paine, R.T. in *Exploitation of Marine Communities* (ed. May, R.M.) (Dahlem Konferenzen, Berlin, 1984).
7. Sissenwine, M.P. *Int. Comm. Northw. Atlant. Fish. Sel. Pap.* 2, 137 (1977).
8. Beddington, J.R. & May, R.M. *Science* 197, 463 (1977).
9. Shepherd, J.G. *Math. Biosci.* 55, 179 (1981).
10. Beddington, J.R. (rapporteur) in *Exploitation of Marine Communities* (ed. May, R.M.) (Dahlem Konferenzen, Berlin, 1984).
11. Wolda, H. *Am. Nat.* 112, 1017 (1978).
12. May, R.M. *Nature* 278, 550 (1979).
13. Beverton, R.J.H. (rapporteur) in *Exploitation of Marine Communities* (ed. May, R.M.) (Dahlem Konferenzen, Berlin, 1984).
14. Shepherd, J.G. in *Exploitation of Marine Communities* (ed. May, R.M.) (Dahlem Konferenzen, Berlin, 1984).

or Fe, and the alloys consist entirely of gamma prime, is that both the strength and the ductility of the alloys are enhanced, whereas usually one of these improvements must be bought at the expense of the other. Once the quenched alloys are annealed, the domains grow (the state of order is improved) and the alloys become both weaker and less ductile. Unfortunately, Inoue *et al.* did not test the variation of the strength of the fine-domain structure with temperature; it is now important to know whether that is also subject to anomalous behaviour.

Another recent Japanese paper, from Tokyo (Ochiai, S., Oya, Y. & Suzuki, T. *Acta metall.* 32, 289; 1984) reports the extent to which third metals can be dissolved in the gamma prime phases Ni_3Al , Ni_3Ga , Ni_3Ge and Ni_3Sn , and the superlattice site preferred by the third metal; these features are related to atomic size ratios and to the various bond strengths. In the present context, it is of particular interest that two of the authors of that paper previously studied the anomalous mechanical behaviour of a number of gamma prime phases (for example, Pt_3In and Pt_3Sn), finding, in general terms, that the more stable the ordered phase the more pronounced the anomalous strengthening with rise of temperature (Suzuki, T. & Oya, Y. *J. mater. Sci.* 16, 2737; 1981). This seems consistent with the orthodox explanation of the anomaly, outlined above.

It should now be possible to answer some very interesting questions by rapid-quenching experiments on such 'model' gamma prime phases, both binary and ternary. First, does the size of the microdomains produced by quenching scale with the stability of the ordered phase? Second, how do the mechanical characteristics of these phases, particularly their anomalous behaviour, react to the introduction of imperfect order — that is, small domains? Finally, what are the consequences of the gradual healing of imperfect order by subsequent heat treatment? In this connection, it is probable that the domain size in a ternary alloy series based, say, on Ni_3Al or on Ni_3Ga , will be a function of concentration of the third element, and if so, it will be illuminating to see how the mechanical anomaly varies across a ternary series prepared by melt-quenching.

In this way, the curious mechanical characteristics of gamma prime can perhaps be related to the mechanical behaviour of imperfectly ordered phases such as Cu_3Au or FeCo , on which a large literature exists. In general, the strength of such alloys peaks at an intermediate degree of order, but the dependence of ductility on the degree of order has not been studied. The introduction of rapid quenching, it seems, may re-open the study of the mechanical characteristics of ordered phases, which has been in the doldrums of late. □

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Blood cell development

The message in the medium

from T.M. Dexter

EVERY minute of life, a human must produce an average of about 150 million red cells and 120 million granulocytes, which constitute the majority of white cells. Mature haematopoietic cells, such as these, are derived from several types of progenitor cell, each committed to develop into only one or two cell lineages; but all progenitor cells take their origin from the same 'stem' cell. The control of this developmental process — the regulation of 'production quotas' and of 'quality control' — is thought to reside in a family of haematopoietic growth factors. To a great extent, these growth factors are still ill-defined molecules present either in serum or in 'conditioned medium' — the fluid left after the growth of certain cells *in vitro*. But the techniques of molecular cloning are beginning to define the factors. One yielded to that approach earlier this year^{1,2}; Gough *et al.*, from Melbourne, report their success with a second factor on page 763 of this issue³. The emerging data are bound to create widespread interest and excitement for developmental biologists.

The significance of these findings is best considered in the context of the overall process of the formation of blood cells which starts during embryogenesis with the laying down of a relatively small number of pluripotent haematopoietic stem cells. By virtue of their ability to self-renew, sufficient stem cells are generated not only to maintain their population throughout adult life but also to produce, by differentiation, the progenitors of all haematopoietic cells. It is obvious that the maintenance of haematopoiesis requires that a balance be maintained between self-renewal and differentiation. If too many stem cells were to differentiate, the system would soon undergo a decline; if too few differentiate, there will not be sufficient input into the progenitor cell compartment to meet the demand.

The progenitor cells, the immediate progeny of the stem cells, are recognized by their ability to undergo proliferation in soft-gel media *in vitro* to produce colonies of mature cells of the appropriate lineage. For many years it has been known that the survival, the proliferation and the development of these 'colony-forming' cells *in vitro* requires the continuous presence of specific growth regulatory molecules.

The story began with independent observations that colonies of granulocytes (neutrophils)⁴ or macrophages⁵ would develop in soft agar, provided that feeder cell layers or conditioned medium were present in the culture. Since then, modifications of this technique have provided the conditions in which the other three myeloid progenitor cell types — those

that spawn red blood cells, platelets and eosinophils — can be stimulated to form colonies (reviewed in ref.6). In all cases, colony development requires the presence of ill-defined growth factors or 'colony-stimulating factors'. Not surprisingly, many workers have formulated (and are still formulating) their own pet names for these factors, causing much confusion both within and outside the field of developmental haematology. Also, there has been a considerable variation in the sources of the factors, the concentrations of factor used and the source of the responding cells (marrow, spleen, fetal liver). Because of this, it has been impossible to determine, for example, whether the common progenitor of both granulocytes and macrophages responds to only one growth factor or to a family of growth factors. Paradoxically, this initial lack of agreement and standardization among the groups involved has been of some benefit, since several different and specific growth factors have been discovered as a result of the variety of starting materials.

The use of conditioned media to facilitate the growth of haematopoietic progenitor cells is still widespread. In the last five years, however, many of the active growth factors have been purified to apparent homogeneity, enabling a clearer picture to emerge of their biological activities and target cells. The properties and the nomenclature (still confusing) of some of the growth factors for myeloid progenitor cells are summarized in the table.

A simple interpretation of the data is that haematopoietic cell development is regulated by a (limited) series of growth factors which are progressively restricted in their biological activities and target cells. Thus, the recently cloned^{1,2} growth factor IL-3 (for definition of abbreviations used, see the table legend) is indifferent to lineage — promoting growth and development of all the different myeloid progenitor cells. Moreover, it facilitates self-renewal of pluripotent (CFU-S) and multipotent (CFC-Mix) stem cells, and can 'immortalize' granulocyte precursors and mast cells *in vitro*. GM-CSF is more restricted, stimulating both proliferation and development of only granulocyte, macrophage and eosinophil colony-forming cells and the proliferation, but not the subsequent development, of the CFC-Mix and the red cell progenitor, BFU-E; to my knowledge, there is no evidence that GM-CSF stimulates self-renewal of any of these cell populations. The growth factors G-CSF and M-CSF are even more restricted in their activities.

Firm evidence that two of these growth factors are indeed quite distinct species

comes from the new data of Gough *et al.*³, who have cloned and sequenced the cDNA of GM-CSF, and deduced the amino acid sequence of GM-CSF from the cDNA sequence. Also of significance is their observation that GM-CSF is encoded by only one gene; hence the reported differences in GM-CSF from different tissues probably reflect post-translational modifications. An intriguing additional finding by the Melbourne group is that, although they show no structural similarity, IL-3 can compete with GM-CSF for binding to its receptor (A.W. Burgess, personal communication). It seems, then, that some multipotential stem cells, progenitor cells and IL-3-dependent cell lines have both IL-3 and GM-CSF receptors; that IL-3 can bind to both receptors, but that GM-CSF can bind only to its own receptor. Since these two regulatory molecules appear to have quite distinct roles vis-a-vis self-

renewal, proliferation and development, it may well be that the outcome of a response depends upon the relative concentrations of the two regulators and the number and accessibility of binding sites. Development may be further regulated by the lineage-restricted molecules G-CSF, M-CSF and erythropoietin.

The problems have by no means all been solved. Nonetheless, the way in which the haematopoietic cell growth factors interact to promote self-renewal, proliferation and development can now be studied in some detail *in vitro*. It will be just as important, of course, to determine whether such growth factors have a role *in vivo*. In the adult, haematopoiesis occurs in the marrow in association with a stromal cell meshwork. Evidence indicates that the stromal cells are not passive bystanders but themselves influence haematopoietic cell development, presumably by supplying the

necessary cell matrix and diffusible molecules⁷. So far, it has been difficult to ascribe to IL-3 or GM-CSF a role in this process — the availability of cDNA probes should now help to resolve this problem.

The question of whether inappropriate expression of these growth factors has a role to play in leukaemogenesis can also now be addressed using the cloned cDNA. With this recent work, haematology has entered a new and exciting era. □

1. Fung, M.C. *et al.* *Nature* **307**, 233 (1984).
2. Tokota, T. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **81**, 1070 (1984).
3. Gough, N.M. *et al.* *Nature* **309**, 763 (1984).
4. Pluznik, D.H. & Sachs, L. *J. cell. comp. Physiol.* **66**, 319 (1965).
5. Bradley, T.R. & Metcalf, D. *Aust. J. exp. Biol. med. Sci.* **44**, 287 (1966).
6. Metcalf, D. in *Tissue Growth Factors* (ed. Baserga, R.) 343 (Springer, New York, 1981).
7. Dexter, T.M. & Allen, T.D. *J. Path.* **141**, 415 (1983).

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Growth Factor	Other Names	Nature of Response	TARGET CELLS						
			CFU-S	CFC-Mix	BFU-E	GM-CFC G ↙ M ↘	Eos-CFC	Meg-CFC	Mast cells
IL-3	BPA HCGF Multi-CSF MCGF PCSF	Self-renewal	+	+	?	+ - ?	ND	ND	+
		Proliferation	+	+	+	+	+	+	+
		Development	ND	+	+	+	+	+	+
GM-CSF	MGI-1GM CSF	Self-renewal	ND	-	-	- -	-	ND	- ?
		Proliferation	ND	+	+	+	+	-	- ?
		Development	ND	-	-	+	+	-	+
G-CSF	MGI-2 D-Factor GM-DF	Self-renewal	ND	-	-	- -	-	ND	-
		Proliferation	ND	+ ?	+ ?	+	+	+	-
		Development	ND	-	-	+	- ?	ND	-
M-CSF	CSF-1 L-cell CSF MGI-1M CSA	Self-renewal	-	-	-	- -	-	-	-
		Proliferation	-	-	-	- ? ++	-	-	-
		Development	-	-	-	- ? ++	-	-	-

BIOLOGICAL ACTIVITIES AND TARGET CELLS OF MYELOID GROWTH FACTORS

The table is compiled from published data and is meant not to be a comprehensive survey of the field but to serve as a reference point for the various names and activities of the growth factors. + indicates a positive response, - a negative response, and ? an equivocal or controversial result. ND, not determined. The full names corresponding to the growth factor acronyms are as follows: IL-3, interleukin-3; BPA, burst-promoting activity; HCGF, haematopoietic cell growth factor; CSF, colony-stimulating factor; MCGF, mast cell growth factor; PCSF, P-cell-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; MGI-1GM, macrophage/granulocyte inducer—granulocyte macrophage; G-CSF, granulocyte colony-stimulating factor; MGI-2, macrophage/granulocyte inducer-2; D-factor, leukaemia cell-differentiation-inducing factor; GM-DF, granulocyte/macrophage and leukaemic cell differentiation-inducing factor; M-CSF, macrophage colony-stimulating factor; MGI-1M, macrophage/granulocyte inducer—macrophage specific; CSA, colony-stimulating activity; CSF-1, colony-stimulating factor-1. The target cells are: CFU-S, colony-forming unit—spleen, multipotential stem cells; CFC-Mix, colony-forming cell—mixed, a multipotential stem cell with the ability to form mixed myeloid colonies in soft agar; BFU-E, burst-forming unit—erythroid, a primitive erythroid progenitor cell assayed *in vitro*; GM-CFC, granulocyte/macrophage colony-forming cell which has an ability to form G (granulocytes), M (macrophages), or mixed colonies *in vitro*; Eos-CFC, eosinophil colony-forming cell; Meg-CFC, megakaryocyte colony-forming cell. BFU-E, GM-CFC, Eos-CFC and Meg-CFC form part of the so-called 'committed' progenitor-cell compartments.

Data analysis

The maximum entropy method

from John Skilling

MAXIMUM entropy is a remarkably powerful and general technique of data analysis. It can be used to sharpen up out-of-focus photographs (as in Fig. 1), to make maps of the radio sky, to generate images from medical scanners, to calculate spectra, to reconstruct electron densities in a crystal and even to determine the positions of fuel rods in a nuclear reactor cooled with liquid sodium. It claims to be applicable whenever one needs to estimate a single vector of proportions $p = (p_1, p_2, \dots, p_M)$ from seriously incomplete data, which could be fitted equally well by many different vectors. It claims to ignore what the proportions represent, which is why it can be applied to so many different inference problems. Maximum entropy always selects the simplest possible result, containing the bare minimum of structure needed to fit the data. Spurious detail is reduced as far as possible, which doubtless accounts for the practical success of the method.

The principle is always the same: one maximizes the entropy $S = -\sum_i p_i \log p_i$ subject to whatever constraints are imposed by the data. The theoretical basis of the method has often been less clear than the practical results however. Now papers by Shore and Johnson (*IEEE Trans. Inform. Theory* **IT-26**, 26; 1980 and **IT-29**, 942; 1983) and by Tikochinsky, Tishby and Levine (*Phys. Rev. Lett.* **52**, 1357; 1984) are making the justification for the maximum entropy method both clear and compelling.

Historically, the use of maximum entropy in data analysis has often been justified by supposing that the proportions were quantized, so that $p_i = n_i/N$. If N quanta were placed 'at random' into M available locations, the relative frequency of sets of occupation numbers n would be degeneracy $N! / \prod n_i!$, which is equivalent

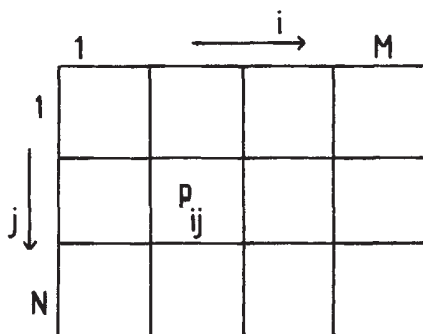


Fig. 2 For definition of i, j, M and N and p_{ij} , see text.

to $\exp(NS)$, at least if N is large. Thus maximum entropy would be the same as maximum degeneracy, which could in turn be identified with maximum probability. Partly because of the dubious status of N and the quantization, this derivation has never been entirely convincing to scientists at large. An alternative justification has been to eschew quantization and identify S directly with (minus) the Shannon information content of p , considered as a vector of probabilities. One can then talk about minimizing the configurational information of p ; but once again the rationale has not proved universally convincing.

The new analyses reach a deeper level that bypasses the earlier derivations, and show that maximum entropy is the only consistent selection procedure. Let us start with Tikochinsky *et al.*, and with a diagram (Fig. 2), in which p_{ij} represents the probability of outcome i when performing experiment j . Tikochinsky *et al.* consider 'reproducible' experiments in which p_{ij} is independent of j , and in which incomplete data are acquired in the form of linear combinations $\langle A \rangle = \sum_i A_i p_i$. Because the data are incomplete (fewer than M com-

binations), the values of $\langle A \rangle$ do not fully determine the values of p . But if we must select just one single result, how should we go about it? Suppose the experiment is repeated N times. We are at liberty to treat each experiment independently. In this case, our selection algorithm (whatever it might be) will choose the same proportions p_i for each experiment j , and these could be combined to give an overall distribution P_i for the outcomes i after the N repetitions. We are also at liberty to treat the whole as one large experiment, in which case our selection algorithm will immediately produce an overall distribution Q_i . Tikochinsky *et al.* point out that we must surely require $P_i \equiv Q_i$ if data from reproducible experiments are to be combined consistently.

Repeating or averaging an experiment does not change its outcome, and it should not change the selected result either. In ten short equations, Tikochinsky *et al.* proceed to prove that the consistency requirements is satisfied if, and only if, the selection algorithm is maximum entropy. In a brilliantly simple way, entropy emerges as an elementary consequence of consistent reasoning.

The consistency approach can be taken even further. Tikochinsky *et al.* couch their analysis in terms of probability distributions, but it often seems a little artificial to have to identify a set of physical proportions with a set of probabilities of outcomes of a random experiment. Proportions and probabilities are, of course, isomorphic, but why should we need to introduce the idea of randomness at all? And why should we need to restrict ourselves to linear data? The answer to both questions is that we need not.

Let us return to Fig. 2, but let p_{ij} now represent the proportion of the total observed quantity assigned to cell i, j of the $M \times N$ array ($\sum_{ij} p_{ij} = 1$): we could be considering a two-dimensional digitized picture. Suppose that the data are some arbitrary functions $U(u)$ and $V(v)$ of the marginals $u_i = \sum_j p_{ij}$ and $v_j = \sum_i p_{ij}$. We are at liberty to ignore the 'north-south' v -structure, and use our selection algorithm to choose an 'east-west' distribution u_i which fits the data U . Similarly, we can select a north-south distribution v_j which fits the data V , without reference to U . Finally, we are at liberty to use all the data, U and V together, to produce immediately an overall distribution p_{ij} . We must surely require $p_{ij} = u_i v_j$ if data from independent experiments are to be combined consistently. North-south data must not interfere with east-west structure, and vice versa. This will be the case if, and only if, the selection algorithm is maximum entropy. Again, entropy emerges as an elementary consequence of consistent reasoning.

This is the derivation of entropy which we at Cambridge now prefer (see our rigorously informal presentation in *Indirect Imaging* ed. Roberts, J.A., Cambridge University Press; 1984, a more mathematical account of which has been

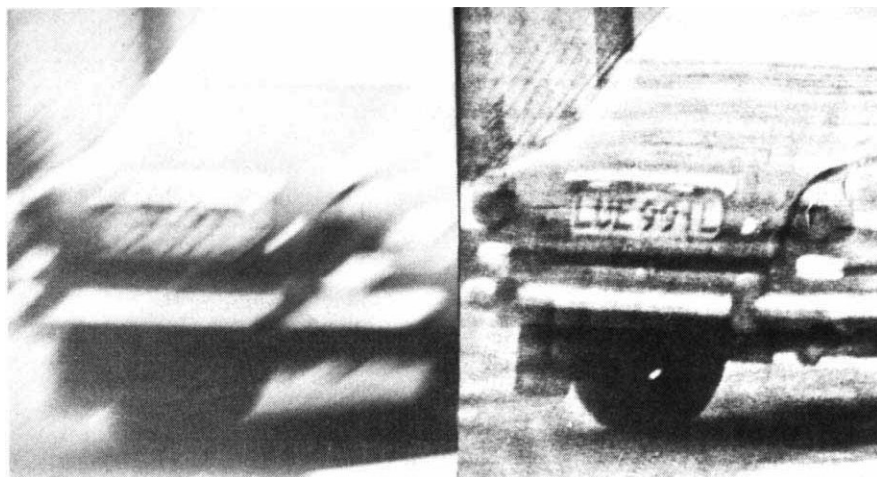


Fig. 1 What maximum entropy techniques can do for car-spotters, including the police.

submitted to *Acta crystallogr. A*). We obtained our derivation from Shore and Johnson's papers, in which the notion of independent dimensions is refined to a requirement of system independence, and the whole treatment is given a proper axiomatic foundation. Shore and Johnson give four axioms — uniqueness, invariance, system independence and subset independence — which must be satisfied by any consistent selection algorithm. In careful and formal work that is remarkably similar to an axiomatic derivation of Shannon information, Shore and Johnson derive the entropy formula, generalized to $S = -\sum p_i \log(p_i/m_i)$ to allow for a possible prior model m . Although they describe p as a probability distribution, this is unnecessary; their work applies equally well to any distribution of proportions, and the technical words 'probability' and 'information' need never appear.

These ideas justify the fundamental claims made for maximum entropy in data analysis, and it is clear that we need not quantify our preference for the maximum entropy selection by anything other than the numerical value of S . It is sufficient to know that we must use maximum entropy — or lay ourselves open to the charge of inconsistency. Let's get on with it. □

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100 years ago

SOME forty years ago Dr. Joule raised the question whether a body that is magnetised undergoes any changes in its temperature; but the question has not yet received a definite solution, the rise of temperature which accompanies magnetisation being ascribed by some to induction currents, and not directly to magnetism. While recognising the influence of the former, Mr. Borgman has tried to show that there is also a change of temperature due to the magnetism and demagnetisation, and that the amount of heat thus disengaged is proportionate to the squares of the temporary magnetism. M. Bachmetieff, having made, at the University of Zurich, an extensive series of experiments, the first part of which is now published in the *Journal of the Russian Chemical Society* (vol. xvi. fasc. 3), arrives at the conclusion that magnetism, *by itself*, produces variations of temperature in magnetised bodies, and that this "magnetic heat" is equal to the product of the magnetic moment by the magnetising force multiplied by a constant; it increases also, within a certain limit, with the frequency of the interruptions of the magnetising current, and increases still more when the direction of the current is alternately changed. Its amount is not equal throughout the length of an iron cylinder, reaching its maximum about its middle and decreasing towards its ends. Its cause must be searched for in purely mechanical forces.

Palaeobotany

Early evolution of leaves

from J.B. Richardson

THE origin and evolution of leaves was a major event in land plant evolution and must have affected all other life on land. Much remains to be learnt, however, of when they first appeared and how they evolved. Fossils described on page 785 of this issue by Gensel¹ confirm what had already been suspected from compression fossils from the Gaspé Peninsula: by the end of the Lower Devonian, vascular plant evolution was quite advanced with complex forms present. The presence of divided non-laminate leaves at the end of the Lower Devonian is already documented² but Gensel¹ describes changes in anatomy in a lateral branch system suggestive of the early evolution of a megaphyll — a laminated leaf with a complex pattern of venation.

Did leaves arise as Zimmermann suggests in his telome theory³, through evolutionary overtopping, planation and webbing? A telome is the most distal dichotomy of a plant. Overtopping describes what occurs if one branch of the dichotomy develops more than the other. The overtopped branches would diminish in size, eventually transforming the equal di-

chotomies into a main stem with lateral branches. Planation describes the change from a three-dimensional arrangement into a single plane of neighbouring telomes. Webbing, the joining of tissues between the lateral branches of the planar telomes, would result in the familiar lamina (leaf-like structure) with dichotomous venation.

This is what Zimmermann proposed but how is the theory testable? One of the restraints on evolutionary hypothesis is the geological record. Do these morphological features appear in the geological succession in the order demanded by the theory? The answer is both yes and no. Simple dichotomously-branched axes have been recorded in the mid-Silurian (Wenlock)⁴, while by the late Silurian, rhyniopsids, with a similar appearance, were common but not diverse. Overtopping was present in the early Devonian (Lower Gedinian)⁵. At that time there was considerable diversity of the rhyniopsids, and zosterophylls, distinguishable from the rhyniopsids on the basis of lateral sporangia, were present⁶. Later, during the Siegenian, the zosterophylls diversified⁷ and the trimerophytes, such as *Dawsonites*, appeared. Later still, in the Lower Emsian of Belgium, a similar flora along with several *Psilophyton* spp. was present⁸ and work by Andrews indicates a high diversity of plants at that time (see *inter alia* ref.9). Trimerophytes in the Emsian exemplify overtopping and reduction of laterals; as Stewart¹⁰ states "Trimerophytosida exhibits almost every branching pattern to be found in megaphyllous vascular plants". Thus, as re-emphasized by Gensel's paper, Zimmermann's leaf-forming processes, apart from planation and possibly webbing, had happened by the late Lower Devonian and much of the geological record supports the telome theory on the origin of megaphylls.

Zimmermann derived a great variety of leaves and leaf-like structures, including microphyllous 'leaves' of the lycopsida, from a rhyniopsid-like ancestor. Bower's enation theory¹¹ is an alternative proposal for deriving microphylls — small leaves with a single central vein. In the telome theory, the microphylls arise by reduction of bunches (trusses) of telomes. Bower's theory is that bumps (enations) emerged from the surface of the stems. As these enations extended (in the evolutionary sense) they developed vascular tissue, first at the base of the enation (as in *Asteroxylon*) and then throughout the enation, as in *Baragwanathia*, which thus became a true microphyll. According to the telome theory this series would be reversed: reduced vascularized microphylls would precede unvascularized outgrowths. Which

RATHER a strange occurrence came recently before my notice, and thinking perhaps you might care to insert it in your columns, I send you the facts of the circumstance. A few days since, towards evening, I killed a snake just close behind my house; it measured about a yard and a half in length, was one of the most deadly of the numerous kinds of snakes found in Java, and bears the name of "Oelar belang." On examining it later I found what I thought to be the tail of another small snake protruding from its mouth, but on pulling it out I was greatly surprised to discover that it was really a snake of the same species, and of almost the same length. There was certainly not more than three inches' difference in the length of the two snakes, and at the time I killed the outside snake only about an inch and a half or two inches of the tail of the one he had swallowed protruded from his mouth. The natives here say that the two snakes must have been fighting, the victor afterwards swallowing his opponent. I should be pleased to know whether such an instance has ever before been brought before your notice, or whether it is really an uncommon case. Soemedang, Java.

M. MONTGIGNY has recently published a pamphlet on the influence of the atmosphere in the apparition of colours seen in the scintillation of stars. He has previously noticed that there is a great predominance of blue in the scintillating colour when rain is approaching, and he is now so convinced of the accuracy of this forecast that it is included among others in the *Bulletin Météorologique* published by the Observatory of Brussels.

From *Nature* 30, 3 July 1884.

theory is correct may seem simple to resolve by accurate age determination of plants whose anatomy is well known. But Stewart¹⁰ proposed at least two lines of lycopsid evolution in the Lower Devonian, one evolving by the enation 'process' and the other according to the telome theory. Earlier, Schweitzer¹² suggested several lines. A great deal of controversy revolves around plants with 'microphylls'. In the Northern Hemisphere the earliest 'microphylls' are on the Gedinian *Drepanophycus*¹³ and 'microphylls' (with no trace of a vein) are known in *Asteroxylon*. Unfortunately many of the main morphological features of these plants, such as sporangia and spores, are unknown or poorly described. In the Southern Hemisphere much controversy surrounds *Baragwanathia* which occurs in Australia in rocks recently re-dated as Upper Silurian (Ludlow). If *Baragwanathia* is now correctly dated and is a true lycopsid then its presence in the Silurian may cast doubts on the origin of the lycopsids from the rhyniophytes.

With the expectation of continued discoveries by those who have already contributed to our knowledge of the early land floras and careful documentation of the geological age of their finds, the next few years promise to be as exciting as, but probably no less controversial than, the recent past. □

1. Gensel, P. G. *Nature* **309**, 785 (1984).
2. Lessuise, A. & Fajon-Demaret, M. *Ann. Soc. géol. Belg.* **103**, 157 (1980).
3. Zimmermann, W. *Palaeobotanist* **1**, 456 (1952).
4. Edwards, D. et al. *Bot. J. Linn. Soc.* **86**, 19 (1983).
5. Edwards D. & Fanning, U. *Proc. R. Soc.* (in the press).
6. Banks, H.P. in *Evolution and Environment* (ed. Drake E.T.) 73 (Yale University Press, 1968).
7. Edwards, D. in *The Caledonides of the British Isles — Reviewed* (eds Harris, A.L., Holland, C.H. & Leake B.E.) 405 (Geol. Soc. Lond., 1979).
8. Gerienne, P. *Ann. Soc. géol. Belg.* **106**, 19 (1983).
9. Banks, H.P. in *Biostratigraphy of Fossil Plants* (eds Dilcher, D.L. & Taylor, T.N.) 1 (Dowden, Hutchinson & Ross, Stroudsburg, 1980).
10. Stewart, W.N. *Paleobotany and the Evolution of Plants* (Cambridge University Press, 1983).
11. Bower, F.O. *Primitive Land Plants* (Macmillan, London, 1935).
12. Schweitzer, H.J. *Bonner Paläobot. Mitteilungen* **7**, 1 (1980).
13. Schweitzer, H.J. *Palaeontographica* **189B**, 1 (1983).

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Immunology

The T-cell receptor completed

from Guil Winchester

T LYMPHOCYTES occupy a central position in the immune response. Like B lymphocytes they are capable of responding to an enormous variety of foreign antigens but they also control and coordinate the activity of other cells in the immune system. It was therefore a source of frustration and embarrassment to immunologists that the surface structure by which T cells recognize antigen remained obstinately invisible for many years. Over the past eighteen months, however, a number of consistent results have gradually been building up into a picture of the T-cell receptor. The publication of the complete sequence of the cDNAs for both subunits of a T-cell receptor on page 757 of this issue¹ completes the task.

The first glimpse of the receptor came last year when monoclonal antibodies, specific for individual T-cell clones, were found to precipitate protein dimers that seemed to be essential for the activation of T cells by antigens^{2,3}. The polypeptide subunits of the dimers have slightly different molecular weights; each polypeptide contains constant regions, which are common to several clones, and variable regions which are clone-specific^{4,6}. The protein chemists were then overtaken by the molecular biologists. In March of this year, two groups simultaneously published the sequence of cDNA clones from both mouse^{7,8} and man⁹ which now seem certain to code for the β -subunit of the receptor (Reinherz cited in ref.1). Since then, a genomic sequence for the β -subunit from mouse has been published¹⁰. The data

show that, like the immunoglobulin gene, the β -subunit gene is split into segments in the germ line and the segments are brought together in differentiated cells to form a functional gene. Since each segment is present in a number of forms, which can be randomly assorted, the genes can encode an enormous number of receptors.

The α -subunit has, however, remained obscure. Now Susumu Tonegawa and his colleagues have isolated and sequenced two distinct cDNA clones from mouse, both of which are expressed and rearranged specifically in T cells¹. Their sequence identifies one as the β -subunit and the other has all the properties predicted for the α -subunit.

The overall structure of the receptor presented by Saito *et al.* looks remarkably like two immunoglobulin light chains, both anchored in the membrane by a stretch of hydrophobic amino acids. Each chain has two domains on the external surface of the membrane, one constant and one variable, with the variable region at the amino-terminal end of the molecule. Between the constant domain and the cell membrane there is a short sequence which contains the cysteine that is thought to bind the two subunits together; this is followed by a sequence that spans the membrane and a short tail found in the cytoplasm. The α -subunit (which is the larger) has a slightly longer tail which contains an extra cysteine. Saito *et al.* suggest that this cysteine may form 'dynamic interactions' with other molecules. One candidate would be the T3 protein which is found in all mature human T cells and which seems to be involved in

the triggering of T cells by antigen (for a review see ref.11). Removal of T3 from the surface (induced by anti-T3 antibodies) is accompanied by loss of the T-cell antigen receptor, implying that they are physically associated.

The new data provide Saito *et al.* with the first opportunity to compare receptor molecules from different T-cell subsets. The earlier clones encoding the β -subunit were isolated from T-helper cells, which activate the various effector cells in the immune system, while the cDNA clones described by Saito *et al.* come from cytotoxic T cells whose primary role is the direct killing of virally infected cells.

Although only the β -subunits from different subsets can be compared, the authors draw two tentative conclusions. One is that the variable-region genes used by cytotoxic and helper cells may well come from different gene pools; the two variable-region genes compared so far are only 20 per cent similar, which is comparable to the degree of similarity between the constant-region genes of the β -subunit of the T-cell receptor and those of the immunoglobulin light chains. Since helper-T cells usually recognize antigen in association with class II major histocompatibility complex (MHC) products, while cytotoxic cells recognize antigen in association with class I MHC products, dissimilarity between the variable regions of helper cells and cytotoxic cells is not unexpected. Moreover, it is compatible with the observation that helper cells and cytotoxic cells have different antigen repertoires.

More surprising is the fact that the β -subunit gene from cytotoxic cells shares a constant-region sequence with one of the helper-cell genes, the identity extending over the entire 3'-untranslated region. Differences between the constant regions of different T-cell subsets might have been expected; in an immunoglobulin molecule the constant region of the heavy chain determines its functional class. Two possibilities that might account for this anomaly are discussed by Saito *et al.* One is simply that functional differences between the two cell types lie on the constant region of the α -chain. The other is that the T-cell receptor is purely a recognition molecule and has a non-specific role in triggering effector functions. This question should be answered as soon as the α -subunit from a T-helper cell has been sequenced. At the rate events are moving in the field that should not be long delayed. □

1. Saito, H. *et al.* *Nature* **309**, 757 (1984).
2. Meuer, S.C. *et al.* *J. exp. Med.* **157**, 705 (1983).
3. Haskins, K. *et al.* *J. exp. Med.* **157**, 1149 (1983).
4. Acuto, C. *et al.* *Cell* **34**, 717 (1983).
5. McIntyre, B.W. & Allison, J.P. *Cell* **34**, 739 (1983).
6. Kappler, J. *et al.* *Cell* **35**, 295 (1983).
7. Hedrick, S.M. *et al.* *Nature* **308**, 149 (1984).
8. Hedrick, S.M. *et al.* *Nature* **308**, 153 (1984).
9. Yanagi, Y. *et al.* *Nature* **308**, 145 (1984).
10. Chien, Y. *et al.* *Nature* **309**, 322 (1984).
11. Beverley, P. *Nature* **304**, 398 (1983).

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Astronomy

Humps and superhumps

from Andrew King

CATAclysmic variables are close binary systems with orbital periods of a few hours, in which a white dwarf accretes matter from a faint low-mass companion star. They have shown themselves to be ideal systems for studying accretion processes, allowing frequent and stringent tests of theoretical ideas. An important new test is reported by Whitehurst, Bath and Charles on page 768 of this issue.

Cataclysmics show convincing evidence of possessing accretion discs; the accreting matter spirals slowly in to the white dwarf through a sequence of Kepler orbits (centrifugal force balancing gravity) under the action of dissipative forces, gradually giving up its gravitational potential energy to radiation. The stability of such a disc is a matter of great importance and under active debate, with dwarf novae providing the test ground. These are a class of cataclysmics which at irregular intervals (weeks to months) brighten dramatically (factors of about 10) in a day or less, remaining at this 'outburst' level for anything up to about 10 days (occasionally longer) before decaying back to their 'quiescent' level in 1 to 3 days. Two types of model have been proposed to explain this behaviour. In one, put forward by G.T. Bath and co-workers (University of Oxford), the 'donor' star (or secondary) is slightly unstable, and at intervals feeds bursts of mass transfer into the accretion disc. In the other, the outburst is a consequence of instabilities purely within the disc, without any change in the state of the secondary: work on these lines is being pursued by groups in Munich, Texas and Santa Cruz (see *Nature* 302, 12; 1983). In their paper on page 768, Whitehurst *et al.* attempt to broaden the debate to include the phenomenon of 'superhumps'.

Superhumps are periodic increases in brightness (of up to 40 per cent) that are observed during the occasional 'superoutbursts', lasting about 12 days, that are additional to the 'normal' outbursts of one subclass of dwarf novae (the SU Ursae Majoris systems). Fascinatingly, the superhumps repeat on a period, P' , that is a few per cent longer than the orbital period, and so cannot be due to some feature locked to the orbital rotation, such as an eclipse, for example. (It is an intriguing question whether the SU Ursae Majoris phenomenon is restricted to short-period dwarf novae, or whether observational selection effects prevent us recognizing it in longer-period systems. See Warner, B. in *Interacting Binaries*, eds Pringle, J.E.P. & Wade, R., Cambridge University Press; in the press, and van Paradijs, J. *Astr. Astrophys.* 125, L16; 1983, for a discussion).

Whitehurst *et al.* focus on two systems which have orbital inclinations high

enough that the secondary star periodically crosses our line of sight to the accretion disc, causing a deep eclipse, repeating at the orbital period, P . Painstaking efforts by B. Warner (Cape Town University) and W. Krzeminski (now Las Campanas) have yielded optical light curves through superoutbursts of these systems. The deep orbital eclipses are still there, indicating that most of the extra superoutburst light comes from the accretion disc. The interesting question which Whitehurst *et al.* address is whether the superhump light is eclipsed. Since the superhumps 'march' slowly through the orbital light curve on the beat period $(1/P - 1/P')^{-1}$ of about 2–3 days, there are phases when the orbital eclipse falls right on top of the superhump. If the superhump light were totally eclipsed at these phases, the residual light at the bottom of the eclipse should be exactly the same as that at phases where eclipse and superhump do not coincide. If, on the contrary, the superhump light were entirely uneclipsed, this residual light should vary in the same manner as the superhump itself. Using data from several eclipses, Whitehurst *et al.* conclude that the latter is the case. This virtually forces the site of the superhump phenomenon to be on the secondary star, as only a rather contrived model could restrict it to the small part of the accretion disc which is never eclipsed.

Activity on the secondary star is, of

course, exactly what is invoked by the Bath model for dwarf nova outbursts; in the disc instability picture such activity would require a separate explanation. Moreover, most models place the superhump site on the accretion disc, where it would be partially eclipsed (see Warner, *op. cit.*). Whitehurst *et al.* suggest that the superhump is due to a bright spot on the secondary where part of the stellar envelope is lost to the white dwarf to cause the superoutburst: the spot is larger than usual since superoutbursts last longer than normal outbursts. The superhump period P' then represents the rotation period of the secondary; that is indeed expected to be just slightly longer than the orbital period P because of the synchronizing effect of tidal torques in a close binary.

All this presupposes that one accepts Whitehurst *et al.*'s conclusion that the superhumps are entirely uneclipsed. Because the superhump does not repeat precisely on each appearance, an escape hatch from this conclusion is still ajar, in that partially eclipsed superhumps may be allowable (see Whitehurst *et al.*'s Fig. 2). Whether they occur is a question that can be settled observationally, and should soon be, with the powerful stimulus of the new paper. But even when this question is disposed of, others will remain, such as why the superhump radiation pattern has such a highly structured and repeating nature. Superhumps and superoutbursts look set to challenge theoreticians and observers for some time to come. □

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Molecular biology

Transposable elements and suppressor genes

from Ian J. Jackson

MOLECULAR biologists like mutations. Determination of the molecular basis of genetic mutations has already revealed unsuspected means by which gene expression can be disrupted. Now a class of mutant genes in species as diverse as yeast, *Drosophila* and mouse is providing more surprises as to mechanisms of mutagenesis, and may well yield information about the regulation of normal development. The mutations are caused by the insertion of transposable genetic elements in or near a gene and are suppressed by recessive genes which map elsewhere in the genome. Recently published work together with data discussed at a meeting in Colorado* reveals a remarkably unified theme among the mutations at the structural level, although the modes of action of the

suppressor genes are proving to be somewhat enigmatic.

The transposable elements that cause the mutations include four classes: Ty of yeast, *copia* and *gypsy* of *Drosophila* and the ecotropic endogenous retroviruses of mouse. Each consists of a few kilobases of DNA flanked at either end by a duplicated sequence of several hundred base pairs, the long terminal repeat (LTR). In all cases where they cause a suppressible mutation, the transposable elements are inserted not into coding DNA but into an intervening or flanking sequence. After insertion, homologous recombination between the two LTRs can result in excision of the internal sequences, leaving a single LTR behind, and often resulting in total or partial reversion of the mutation. In all three species this occurs at a frequency of about 10^{-5} – 10^{-6} per generation. Suppression of these

* UCL.A Symposium, 'Genome rearrangement', held at Steamboat Springs, Colorado, 8–12 April 1984.

mutations, however, is not caused by an increase in the excision rate of the element. Instead, in some cases at least, it seems that the mutant phenotype is a result of transcription of the inserted element, and it is on this transcription that the suppressor genes act. All four classes of element have a promoter of transcription in one LTR and a terminator in the other, and thus will produce, under the right circumstances and where there has been no internal excision, a RNA molecule running the full length of the element. Whether, in all cases, this transcription causes the mutant phenotype remains to be seen, but the nature of the suppression and the wild-type function of the suppressor genes should prove revealing.

One good example of the phenomenon is the production of the auxotrophic mutations *his4-912* and *his4-917* in yeast by insertion of a Ty element upstream of the *HIS4* gene¹. The *spt3-* mutation (formerly *spm3-*) of yeast suppresses *his4-917*, in which transcription of the Ty is away from the *HIS4* gene, but it does not suppress *his4-912*, in which transcription is towards *HIS4*. However, excision of most of Ty leaving behind a solo δ -sequence (the LTR of Ty) results in a cold-sensitive mutant phenotype which is then suppressible by *spt3-*. This arrangement has allowed Gerry Fink (Whitehead Institute/MIT) to examine the expression of the gene. At low temperatures, transcription of the *HIS4* gene initiates from the δ -sequence, 5' of the gene, instead of the correct site. The resultant RNA is inactive. In the presence of *spt3-*, transcription starts from the correct position at all temperatures and all transcription from the normal origin in all δ -sequences of the cell is inhibited. Thus the simplest explanation for the mechanism of suppression is that transcription from the δ -sequences of the Ty elements placed in critical regions 5' of a gene will prevent normal gene expression, and that *spt3-* mutants lack the ability to transcribe some or all of the Tys, so relieving the mutation. In this model the wild-type *SPT3* gene encodes a transcription factor required for initiation of transcription from δ -sequences. Perhaps the Ty mutations that are not suppressed by the *spt3-* mutation are transcribed by a different mode.

Inevitably, the genetics of the *Drosophila* suppressors is much more complex and the molecular biology less clearcut. The suppressor of hairy wing gene, *su(Hw)*, not only suppresses hairy wing, but a number of alleles at other loci, including *cut*, *scute*, *lozenge*, *forked*, *rudimentary* and several alleles of the homoeotic complex, *bithorax*. In work published last year, Modolell, Bender and Meselson reported the cloning of DNA from five suppressible alleles of *bithorax*². Each of them contained the same transposable element, *gypsy*; indeed, all the genes affected by *su(Hw)*, except *rudimentary*, contained *gypsy* elements. Modolell *et al.*

suggested that the product of the wild-type *su(Hw)* gene somehow interacts with *gypsy* to cause the mutations; a mutation at *su(Hw)* prevents this interaction and so suppresses the mutagenic effect of *gypsy*. At the Colorado meeting, Welcome Bender (Harvard University) presented data from *bithorax* which indicate that it may be transcription of the *gypsy* element which is causing the mutant phenotype. It cannot simply be the effect of insertion of *gypsy* into *bithorax* because other insertions at the same locations have a less pronounced effect. Similarly, chromosomal breaks have a different effect from *gypsy* insertion, suggesting that premature termination in the element is not to blame. Bender has gone on to show that in the presence of homozygous *su(Hw)*, transcription of the total population of *gypsy* elements is reduced fivefold. Whether the reduction of transcription is more from certain elements than from others is not known, but an effect on gene expression via transcription of the element is certainly strongly suggested. The problem remains of why *su(Hw)* suppresses two alleles of *rudimentary* which do not contain *gypsy* sequences.

The precise mode of action of the *su(Hw)* suppressor gene and the function of the product of its wild type have not emerged, but the observations of Paul Bingham (SUNY, Stonybrook) in relation to the *white-apricot* gene of *Drosophila* may be enlightening. The *white-apricot* (w^a) phenotype is caused by insertion of the transposable element, *copia*, into an intervening sequence of the *white* eye-colour gene (refs 3, 4 and K. O'Hare, personal communication). The effect of the w^a gene is suppressed by *suppressor of white-apricot*, *su(w^a)*, which results in a darker eye colour, but can also be enhanced to produce a paler eye-colour by *suppressor of forked*, *su(f)*. Somewhat enigmatically, *su(f)* also suppresses the same alleles of *forked* and *lozenge* that are suppressed by *su(Hw)* and contain *gypsy* insertions. Bingham reported that a partial revertant of w^a , containing a single LTR insertion in place of the whole *copia*, was not affected by either *su(w^a)* or *su(f)*. This again suggests some effect of transcription of the element on the gene into which it has been inserted. This is further supported by evidence that the very low level of normal *white* transcript produced from the w^a gene was reduced still further in the presence of *su(f)*, but increased several fold when *su(w^a)* was present (Gerry Rubin, University of California, Berkeley).

To investigate the nature of suppression and the normal function of the suppressor genes further, Bingham has made several new mutations at *su(w^a)*, almost all of which are recessive lethals. The exception, when homozygous, produces a fly that lacks wings and halteres, in a manner which suggests that specific cells of the imaginal discs die or fail to develop. The normal function of *su(w^a)*, suggests Bingham, is to

provide developmental signals within *Drosophila*. How the *copia* element in the w^a gene responds to these signals in such a way as to restore a normal white phenotype is a mystery. A guess would be that in certain cells the element is dependent for transcription upon these developmental signals, and that some mutations of the signal prevent the expression of this particular *copia*, and thus suppress w^a , whilst still allowing normal development of the organism.

Hope Sweet, of the Jackson Laboratories, reported last year the identification of the first recessive suppressor mutation in the mouse⁵. The gene, *dilute suppressor* (*dsu*), suppresses the *dilute* mutation (*d*). *Dilute* produces a pale coat colour. Jenkins *et al.* have shown it to be due to the insertion of an endogenous retrovirus into the mouse genome^{6,7}. Revertants that are left with only one of the two LTRs have normal coat colour. Jenkins (University of Cincinnati) reported that the retrovirus seems to be inserted into a noncoding segment of DNA, so the means by which the retrovirus disrupts expression of the *dilute* gene are not evident. Perhaps the presence of the intact viral DNA prevents initiation, or causes premature termination or incorrect splicing of the *dilute* transcript. Although mice bearing the retrovirus do not become viraemic, the viral promoter might be active in melanocytes in a way that prevents correct *dilute* expression and, so, coat colour.

The effect of *dsu* on other genes may help explain how it suppresses *d* and, in turn, the cause of the *d* mutation. An allele of *d*, *dilute-lethal* (*d'*), causes both a pale coat and a fatal neurological disorder. The coat coloration effect but not the lethality of *d'* is suppressed by *dsu*. Hope Sweet (personal communication) has also shown that *dsu* suppresses *ashen*, a mutation which mimics the phenotype of *dilute*. Neither *d'* nor *ashen* is associated with a retrovirus of the same type that causes *dilute*, although the presence of another virus or solo LTR has not been excluded. It seems, however, that the action of *dsu* is involved more directly in affecting the melanocyte morphology which gives rise to the *dilute* or *ashen* phenotype than in the expression of the genes themselves. How a recessive suppressor could act in this way, and the function of the wild-type *dsu*, are of considerable interest; experiments in progress at Cincinnati and the Jackson Laboratories should illuminate these points. □

1. Roeder, G.S. & Fink, G.R. in *Mobile Genetic Elements* (ed. Shapiro, J.A.) 300 (Academic, New York, 1983).
2. Modolell, J., Bender, W. & Meselson, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1678 (1983).
3. Bingham, P.M. *et al. Cell* **25**, 693 (1981).
4. O'Hare, K.M., Lewis, R. & Rubin, G.M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6917 (1983).
5. Sweet, H.G. *J. Hered.* **74**, 305 (1983).
6. Jenkins, N.A. *et al. Nature* **293**, 370 (1981).
7. Copeland, N.G. *et al. Cell* **33**, 379 (1983).

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A large-scale isotope anomaly in the Southern Hemisphere mantle

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Basalts from many Southern Hemisphere regions have anomalous Sr and Pb isotopic characteristics. This article shows that the isotopic mantle anomaly is globe-encircling in extent, centred on latitude 30° S. Arguments suggesting that this mantle anomaly has been in existence for billions of years place severe constraints on mantle convection models.

RECENTLY, Dupré and Allègre¹ made an important observation concerning the existence of a large domain (10⁸ km²), centred in the Indian Ocean, which is delineated by oceanic island and oceanic crust basalts having anomalous Sr, Nd and Pb isotopic signatures. The existing isotopic data for young basalts show that this isotopic mantle domain, here termed the Dupal¹ anomaly, can be traced nearly continuously around the Southern Hemisphere between the Equator and 60° S. Part of this anomaly was previously delineated in the central Pacific². This is the largest mantle geochemical domain yet delineated; I argue that this isotope domain was produced early in Earth history by core-mantle-crust differentiation processes. Survival of this anomaly strongly constrains models of mantle convection.

Isotope criteria

Three criteria are used to quantify the Dupal anomaly: (1) ²⁰⁷Pb/²⁰⁴Pb and (2) ²⁰⁸Pb/²⁰⁴Pb deviation from a Northern Hemisphere reference line (NHRL), (3) absolute value of ⁸⁷Sr/⁸⁶Sr. Choice of a Northern Hemisphere Pb reference line is arbitrary, but convenient because of the abundance of data and essential colinearity of mid-oceanic ridge basalt (MORB) and oceanic island basalt (OIB) data on Pb-Pb plots. The reference lines were drawn to include MORB (both N-type and E-type), the islands of Hawaii, Iceland, Azores, Canaries and Cape Verde³, and the north-east Pacific seamounts⁴. The equations of the adopted reference lines (NHRL) are:

$$^{207}\text{Pb}/^{204}\text{Pb} = 0.1084(^{206}\text{Pb}/^{204}\text{Pb}) + 13.491$$

$$^{208}\text{Pb}/^{204}\text{Pb} = 1.209(^{206}\text{Pb}/^{204}\text{Pb}) + 15.627$$

The magnitude of the isotopic anomaly of any given data set (DS) is then expressed as the vertical deviation in ²⁰⁷Pb/²⁰⁴Pb or ²⁰⁸Pb/²⁰⁴Pb from these reference lines:

$$\Delta 7/4 = [(^{207}\text{Pb}/^{204}\text{Pb})_{\text{DS}} - (^{207}\text{Pb}/^{204}\text{Pb})_{\text{NHRL}}]100$$

$$\Delta 8/4 = [(^{208}\text{Pb}/^{204}\text{Pb})_{\text{DS}} - (^{208}\text{Pb}/^{204}\text{Pb})_{\text{NHRL}}]100$$

For ⁸⁷Sr/⁸⁶Sr, I use the absolute magnitude of the ratio, expressed as:

$$\Delta \text{Sr} = [(^{87}\text{Sr}/^{86}\text{Sr})_{\text{DS}} - 0.7]10^4$$

The coherence between these three anomaly criteria is fairly good. Figure 1 shows the correlation between $\Delta 7/4$, $\Delta 8/4$ and ΔSr . The limiting precision of these criteria is that derived from the isotopic measurements themselves, and is largest for $\Delta 7/4$ because mass spectrometer fractionation effects are large relative to the total variation of $\Delta 7/4$ (measurement precision of ± 2 units is 10% of total $\Delta 7/4$ variation of 20 units) and the fractionation vector is steeply inclined to the reference line. The $\Delta 8/4$ criterion is more robust because the fractionation vector is semi-parallel to the reference line and the total spread in $\Delta 8/4$ is large (measurement precision of ± 5 units is only 3% of the total $\Delta 8/4$ variation of 190 units). Likewise, the measurement precision of ΔSr is small with respect to the observed spread. There are, of course, significant and unconstrained sources of

uncertainty due to 'geological scatter'—the fact that averaged island data sets are used which may show large isotopic variations internally. For data sets involving many samples (Iceland, Hawaii, Galapagos), this scatter may be effectively averaged out; for some data sets, however, only one or two analyses are available and the problem of representativeness may be very serious. Note, however, that the variations of Pb isotopic ratios within a given island basalt suite are commonly sub-parallel to the reference lines, so that the calculated Δ values are insensitive to this kind of intra-island isotopic heterogeneity.

Data

The basic data set used is that compiled by Zindler *et al.*³; these data have been augmented with data from other studies, including some basalts from continental environments. The basic criterion has been to restrict the data sets to basaltic rocks and, for the continental environments, I have used only basalts for which Pb data exists, and which carry ultramafic mantle xenoliths (as a precaution against crustal contamination). These continental basalts are included because they seem to fit the picture, and fill in several important geographical gaps. Several of the basalt suites may be related to subduction zones (Pribilof's and Fiji); in the case of Fiji, only the young alkali basalt suite was used, as this is claimed to postdate subduction involvement⁴. Several suites range up to 85 Myr in age (Walvis Ridge, Rio Grande Rise, Ninety-East Ridge, New England seamounts); while the age correction will not significantly affect the anomaly criterion, it does affect the geographical position of these suites. (When formed, the Ninety-East Ridge samples were located some 40°–50° south of their present position.) Inclusion of these older suites shows that the Dupal anomaly has been a persistent feature of the mantle for at least 10⁸ yr. Table 1 summarizes the data suites used, not including the Pacific ocean Sr data of Hedge² and Duncan and Compston⁵.

World maps of these data, contoured for the three anomaly criteria, are shown in Fig. 2. While the configuration of the Dupal anomaly varies between the three different anomaly criteria, it is, nevertheless, a striking feature on all three maps, occupying a band of some 60° of latitude centred on 30°–40° S. The strongest maximum in the anomaly for all three criteria stretches from the southern mid-Atlantic Ridge to the central Indian ocean; a second maximum shows up on the ΔSr and $\Delta 8/4$ plots in the central Pacific. Very few Pb data have yet been published for this region. Based on available isotopic data (both Pb and Sr)^{2,5–9}, it appears that a configuration similar to the steep gradient between the islands of Tristan, Gough and St Helena in the Atlantic may exist, with Samoa, Raratonga and the Marquesas Islands reflecting a Dupal signature and the Tubuai Islands and southern Tuamotu Islands showing low anomaly values, similar to St Helena. Note that these two regions are almost antipodal to each other, with a near-equatorial two-fold axis of symmetry located at $\sim 100^\circ \text{E}$.

Aside from the central Pacific, the other areas where there is little data coverage are the high latitudes; these regions are

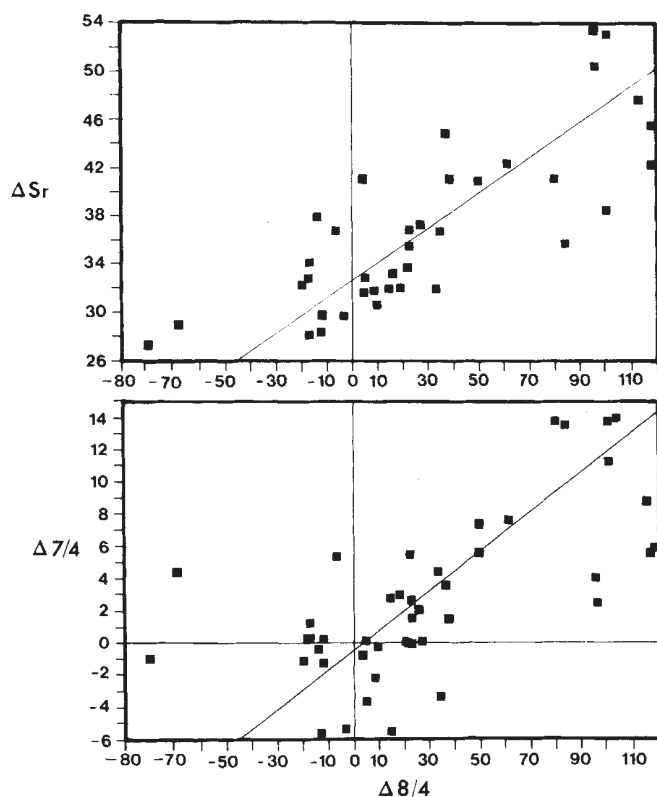


Fig. 1 Anomaly criteria ΔSr and $\Delta 7/4$ plotted against $\Delta 8/4$; data from Table 1. See text for definition of anomaly criteria. Best fit regression lines are: $\Delta Sr = 32.4 + 0.146(\Delta 8/4)$, $r = 0.85$; and $\Delta 7/4 = -0.86 + 0.127(\Delta 8/4)$, $r = 0.69$.

apparently characterized by unusually low ΔSr and negative values of $\Delta 7/4$ and $\Delta 8/4$. There is a good correlation between the features of the isotopic anomaly patterns of Fig. 2 and the degree-2 features of the Earth's geoid¹⁰. The geoid shows negative 'caps' in the polar regions and a nearly continuous belt of positive values centred on the Equator. The degree-2 geoid has been related by Busse¹¹ to a quadrupolar convection regime in the mantle, with lower mantle upwelling centred on an equatorial axis of symmetry. The isotopic anomaly data also suggest a simple quadrupolar pattern, but one which is more nearly axisymmetric with the Earth's spin axis. Higher-degree features of the isotopic anomaly pattern, such as the antipodal St Helena-type lows, have no obvious counterpart in the geoid.

Note that some complexities exist in the isotopic data for Hawaii and the Azores which have been obscured here by using averaged data. The Koolau series, Oahu¹² and the eastern end of Sao Miguel, Azores^{6,13,14}, show rather prominent Dupal signatures, with $\Delta Sr = 41$ and 53, and $\Delta 8/4 = 51$ and 44, respectively.

Implications

In evaluating models for the origin of this anomaly, several features need to be considered: (1) the fact that the anomaly is present in both OIB and MORB data (at least in the Indian Ocean); (2) the approximate coherence of the three anomaly criteria; and (3) positive values of the anomaly criteria require high time-integrated values for the parent/daughter ratios in the source (in the case of $\Delta 7/4$, the required high U/Pb value must, in addition, date back to early in Earth history), and (4) evidence for the Dupal signature in the past geological record. These points will be considered individually.

(1) Dupré and Allègre¹ proposed that the MORB reservoir (upper mantle) is polluted by material from the OIB reservoir (lower mantle), thereby transferring the anomaly signature from the lower to the upper mantle without creating complete isotopic

identity between the two reservoirs. One can also conceive of a non-layered mantle scenario, where the OIB reservoirs are blobs distributed through the MORB reservoir and where partial cross-pollution takes place during partial melting and subsequent high-level magmatic processes^{3,15}.

Another explanation would relate these two reservoirs through a common parentage. If the depleted MORB reservoir is created by extraction of continental crust¹⁶⁻¹⁹, and if the Dupal anomaly signature predates the formation of the MORB reservoir, then the existence of the anomaly signature in the MORB reservoir could simply be inherited from its mantle precursor. To be viable, this mechanism would require that the various MORB and OIB reservoirs have maintained their relative lateral positions over long periods of geological time, which seems unlikely in view of the rapid convective motions of the upper MORB reservoir. I prefer the 'pollution' explanation advanced by Dupré and Allègre¹.

(2) As shown in Fig. 1, the three anomaly criteria are reasonably, though not perfectly, coherent (r ranges from 0.69 to 0.85). Each anomaly criterion depends essentially on the time-integrated parent/daughter ratio in the reservoir; their coherence therefore suggests that the Dupal reservoir has higher time-integrated values of Rb/Sr (ΔSr), Th/Pb ($\Delta 8/4$) and $^{235}\text{U}/\text{Pb}$ ($\Delta 7/4$) than the reference reservoir. Because of the coupling of U/Pb and Th/Pb in the $^{208}\text{Pb}/^{204}\text{Pb}$ – $^{206}\text{Pb}/^{204}\text{Pb}$ plot, the high $\Delta 8/4$ also requires high Th/U ratios. In other words, the Dupal source is characterized by high relative Rb/Sr, $^{235}\text{U}/\text{Pb}$ and Th/U; in most petrogenetic processes, the numerator element in these ratios is observed to behave more incompatibly than the denominator element, so that the approximate coherence of the three anomaly criteria is not surprising.

(3) The Dupal reservoir is characterized by relatively high time-integrated values of Rb/Sr, $^{235}\text{U}/\text{Pb}$ and Th/U. What does this imply for development of the Dupal reservoir? One obvious source for high Rb/Sr, U/Pb and Th/U material is the continental crust, and in fact, Dupré and Allègre¹ favoured the subduction of sediments into the mantle to explain the anomalous isotopic characteristics of the Indian ocean Dupal island suite. Other authors^{3,20-22} have argued for reinjection of oceanic crust or sediment to provide a component in many or all OIBs.

Recent (<500 Myr) injection of crustal material is not a satisfactory mechanism for production of the Dupal isotopic signature, as this would produce high angle Pb isotope arrays, with an apparent origin somewhere along the Northern Hemisphere reference array. With the exception of Tristan, intra-suite Dupal trends appear instead to be subparallel to the reference array (for example, Gough, Walvis, Bouvet, Kerguelen). Furthermore, the suites showing the most marked Dupal signature define an inter-suite Pb–Pb array which is parallel to the reference array, but shifted to higher $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ values. This array, which is defined by 90° E-ridge—Discovery Seamount, Gough, St Paul, Crozet and Amsterdam—covers a large range in $^{206}\text{Pb}/^{204}\text{Pb}$ (17.9–19.2), and cannot be generated by contamination of reservoirs lying along the reference array with crustal material, even if that were heterogeneous. Inclusion of Sr isotope data only enhances the implausibility of this mechanism, as $^{87}\text{Sr}/^{86}\text{Sr}$ shows no significant correlation with position along this 'upper bound' array.

This problem can be circumvented if the crustal contaminant was reinjected into the mantle a long time ago, before the processes which have subsequently produced the 'lineation' of the reference suite along an apparent 1,700-Myr isochron, and which are also responsible for the subparallel lineation of the Dupal suites. Several reinjection models^{3,21,22}, in fact, propose that ancient oceanic lithosphere is responsible for the isotopic signature of many OIB suites, though it has yet to be demonstrated that such material will have the requisite high U/Pb and Th/U ratios. Furthermore, enhanced U/Pb ratios in altered oceanic crust should be accompanied by enhanced U/ ^3He ratios, which would lead in several billion years to markedly enhanced $^4\text{He}/^3\text{He}$ ratios; there is no obvious difference in He isotope ratios between Dupal and non-Dupal islands²³.

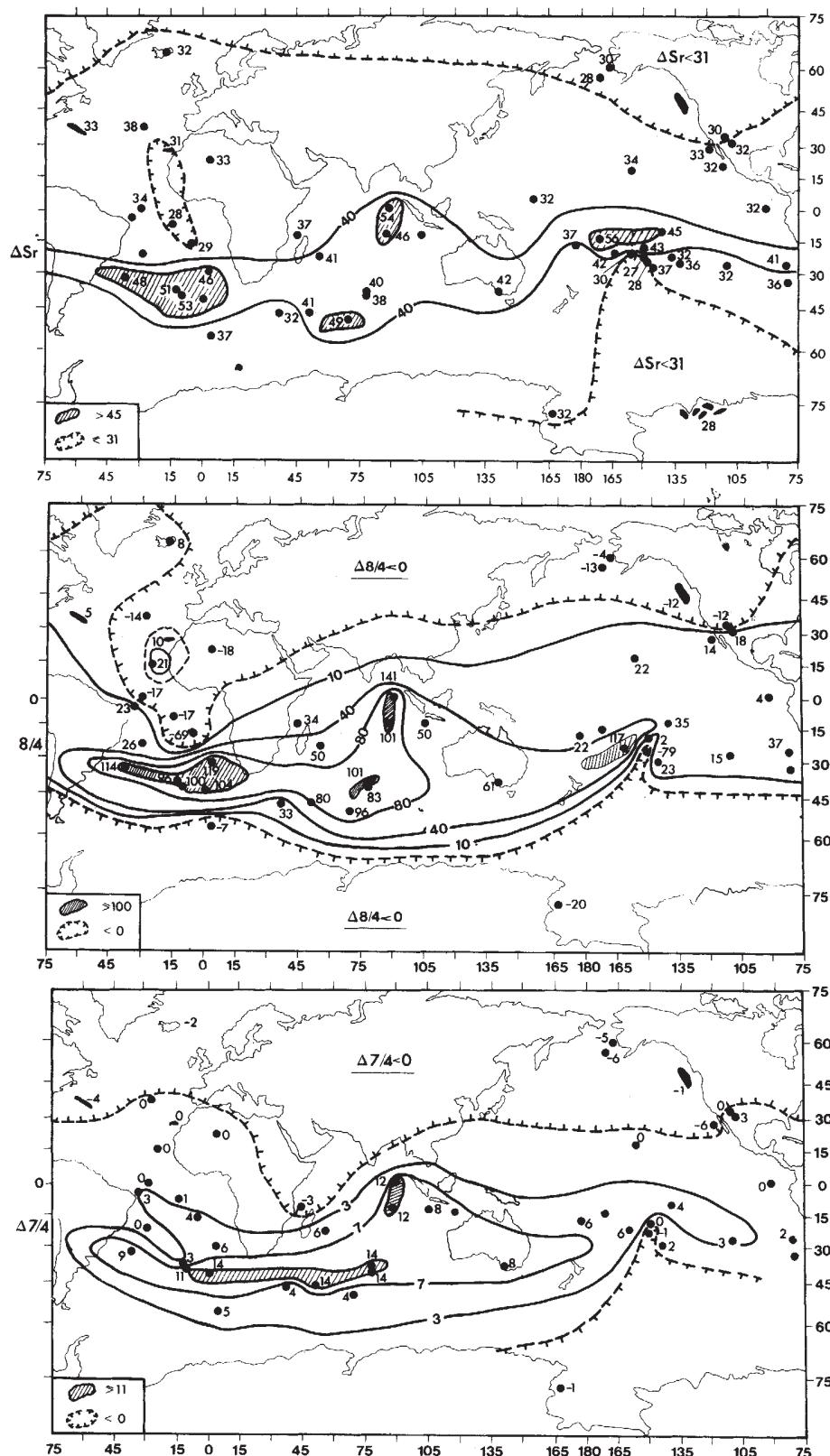


Fig. 2 World maps (Miller cylindrical projection) showing distribution of the ΔSr (upper), $\Delta 8/4$ (middle) and $\Delta 7/4$ (lower) anomaly criteria; contours are fitted by eye and are subjective in many areas of non-existent data. Anomaly data are from Table 1, augmented, in the case of ΔSr , by data from the Pacific Ocean^{2,5} and Antarctica⁴⁶. Also shown on all three maps are data for the Ninety-East Ridge¹, although these are samples of older age and should be plotted at a more southerly position consistent with their location of origin.

It has also been suggested that ancient sub-continental lithosphere may delaminate and provide the necessary 'enriched' reservoir²⁴. This mixing would have to have occurred early in Earth history for the reasons discussed above. In any event, these reinjection models will need to address the fact that the Dupal anomaly is a coherent geographical domain, and is not randomly dispersed amongst the various OIB locations.

I present the following alternative hypothesis to the reinjection model of the Dupal anomaly, as a stimulus for further investi-

gation. First, the anomaly is related to very early ($>3,000$ Myr) development of U/Pb, Rb/Sr and Th/U enrichments in the Dupal source mantle relative to the reference mantle. Second, this relative enrichment either derives directly from constructional heterogeneities in the initial Earth, or is related to heterogeneities developed during early stages of continental crust extraction. (More crust was extracted from the reference mantle reservoir, causing it to be more depleted than the Dupal mantle reservoir.) Third, in common with most other oceanic Pb models,

Table 1 Sr and Pb isotope ratios, averaged by island or area

	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$\Delta 7/4$	$\Delta 8/4$	Ref.
Ahaggar	19.915	15.653	39.525	0.70328	0.3	-17.9	29
Amsterdam (1)	19.170	15.708	39.810	0.70400	13.9	100.6	1
Ascension	19.568	15.625	39.112	0.70282	1.3	-17.3	3
Azores	19.731	15.626	39.341	0.70379	-0.4	-14.1	3
Bouvet (3)	19.445	15.652	39.065	0.70369	5.3	-7.1	3
Canaries	19.600	15.613	39.419	0.70307	-0.3	9.6	3
Cape Verde (2)	19.080	15.560	38.900	—	0.1	20.5	30
Christmas	18.896	15.616	38.972	—	7.7	50.0	*†
Comores (2)	19.300	15.549	39.300	0.70368	-3.4	33.9	1
Crozet (1)	18.913	15.679	39.290	0.70411	13.8	79.7	1
Discovery (1)	18.246	15.609	38.723	—	14.0	103.6	30
Easter (2)	19.200	15.600	38.985	0.70320	2.8	14.5	3
Fernando d'Noronha (2)	19.501	15.631	39.429	—	2.6	22.5	30
Fiji	18.688	15.572	38.442	0.70369	5.5	22.1	4
Galapagos	18.953	15.547	38.586	0.70317	0.1	4.5	3
Gough	18.435	15.599	38.917	0.70532	11.0	100.2	3
Guadalupe	20.151	15.620	40.134	0.70326	-5.5	14.4	30
Hawaii	18.338	15.479	38.016	0.70338	0.0	21.8	3
Iceland	18.752	15.502	38.382	0.70317	-2.2	8.4	3
Kerguelen	18.188	15.503	38.573	0.70492	4.0	95.7	3
Marion-Prince Edward	18.570	15.548	38.412	0.70320	4.4	33.4	*‡
Marquesas	19.107	15.597	39.077	0.70451	3.5	35	8
NE seamounts	20.458	15.672	40.406	0.70329	-3.7	4.5	31
NE Pacific seamounts	18.575	15.492	37.966	—	-1.3	-11.8	32
Nunivak (1)	18.628	15.457	38.112	0.70297	-5.3	-3.6	33, 34
Potrillo (1)	18.541	15.530	38.223	0.70320	2.9	18.0	34, 35
Pribilofs	18.912	15.485	38.364	0.70284	-5.6	-12.8	36
Rapa (3)	19.051	15.578	38.892	0.70370	2.2	23.3	2, 9
Raratonga (2)	18.338	15.536	38.973	0.70420	5.7	117.5	2, 9
Reunion (2)	18.800	15.585	38.850	0.70410	5.6	49.4	1, 37
Rio Grande Rise (1)	17.619	15.490	38.073	0.70476	8.9	114.5	*§
Ross	19.870	15.634	39.450	0.70323	-1.1	-20.0	38, 39
San Carlos (1)	18.591	15.508	37.981	0.70298	0.2	-12.3	34, 35
San Felix (1)	19.312	15.602	39.349	0.70409	1.8	37.4	*
St Helena	20.804	15.789	40.091	0.70293	4.3	-68.8	3
St Paul (1)	18.684	15.653	39.050	0.70383	13.7	83.4	1
St Paul's Rocks (1)	19.773	15.638	39.360	0.70341	0.4	-17.3	40
Tahiti	19.006	15.547	38.624	0.70411	-0.4	1.9	5, 9
Trindade	19.069	15.559	38.945	—	0.1	26.4	41
Tristan da Cunha	18.551	15.527	39.013	0.70507	2.5	95.8	3
Tubuai	21.104	15.770	40.354	0.70277	-0.9	-78.8	8
Walvis Ridge	17.914	15.492	38.472	0.70457	5.9	118.7	42
W. Victoria	18.444	15.567	38.540	0.70425	7.7	61.4	43, 44

Also utilized are Sr isotope data on Gough, St Paul, Kerguelen, Amsterdam (S.R.H. and C. Brooks, unpublished data), and Kerguelen, Easter, Guadalupe, Gough, Tristan and St Helena⁶. Numbers in parentheses after locality are the least numbers of analyses involved in cases for which $n < 4$. Pb data from ref. 9 has been normalized to absolute values using California Institute of Technology Pb standard data of ref. 45.

* Unpublished data from S.R.H. and † J. Morris, ‡ A. J. Erlank, § H. Staudigel, || D. Gerlach.

some means is required to produce the apparent secondary isochron Pb-Pb arrays observed in intra-island basalt suites, and in the reference reservoir suites as a whole. This requires a process, with a mean age of ~2,000 Myr, that will increase the U + Th/Pb ratio of all oceanic mantle sources. Core-pumping of Pb has been advocated for this¹⁶, as has concentration of Pb into the lower crust¹⁹. Mixing with ancient high U/Pb oceanic crust (with present $^{206}\text{Pb}/^{204}\text{Pb} \geq$ that of St Helena) has also been advocated³. I have little to add to the problem of the Pb paradox, other than my prejudice that the Dupal anomaly predates, and is perhaps not even related to, the events which have created the oceanic Pb paradox.

(4) As outlined, the Dupal anomaly is globe-encircling and restricted to the Southern Hemisphere. I have argued above that this anomaly has probably existed as a mantle entity for billions of years.

Support for the antiquity of this anomaly comes from Sinha²⁵ who compiled existing Pb isotope data for continental rock and ore leads of all ages, and showed that samples from the Southern Hemisphere fall along a higher μ growth curve than did those from the Northern Hemisphere (closed system μ s of 9.3 and 8.9 respectively). This distinction was present even in the oldest rocks considered (>3,000 Myr). This hemispheric distinction is not apparent in Pb data from conformable ore deposits^{26,27} but

many of these leads appear to be generated in an arc setting and may involve rather extensive mixing in sedimentary environments. Sinha did not compile $^{208}\text{Pb}/^{204}\text{Pb}$ data, so the existence of a $\Delta 8/4$ anomaly in this continental data set is not verifiable at present.

Note that the rock Pb isotopic data sets compiled by Manhes *et al.*²⁸, also show the Sinha divergence. The eight Southern Hemisphere data sets have a higher average $^{207}\text{Pb}/^{204}\text{Pb}$ intercept value than do the 12 Northern Hemisphere data sets. These sets consist of data of variable analytical quality, and represent a diversity of rock types with both crustal and mantle affinities. If the Sinha hemispheric Pb divergence withstands further scrutiny, two important conclusions are implied: (1) if this divergence is genetically related to the Dupal anomaly, then the Dupal anomaly is indeed of ancient constitution and; (2) the existence of a Dupal-type anomaly in continental rocks suggests a genetic link between oceanic island mantle and continental crust. Because the continental crust is presumed to derived from the mantle, it could carry the Dupal signature with it; on the other hand, if the Dupal anomaly originated by the ancient reinjection of continental material, the signature may have been originally bred in the Gondwana crustal entity and have been inoculated into the subjacent mantle during later crustal reinjection.

Future directions

The Dupal anomaly is defined on the basis of the present position of the oceanic islands and continents. If the anomaly is of ancient parentage where were these continental blocks and oceanic reservoirs in the past? Have they been anchored to the Southern Hemisphere? Could there be a mantle convection regime which would not, in billions of years, destroy the isotopic signature of the Dupal mantle, and which would not decouple the geographical positions of the Dupal continents and mantle reservoirs?

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1. Dupré, B. & Allegre, C. J. *Nature* **303**, 142–146 (1983).
2. Hedge, C. E. *Earth planet. Sci. Lett.* **38**, 88–94 (1978).
3. Zindler, A., Jaouitz, E. & Goldstein, S. *Nature* **298**, 519–523 (1982).
4. Gill, J. B. *Earth planet. Sci. Lett.* (in the press).
5. Duncan, R. A. & Compston, W. *Geology* **4**, 728–732 (1976).
6. White, W. M. & Hofmann, A. W. *Nature* **296**, 821–825 (1982).
7. Tatsumoto, M., Unruh, D. M., Pettingill, H. S., Basu, A. R. & Barszczus, H. G. *EOS* **64**, 348 (1983).
8. Vidal, Ph., Chauvel, C. & Brousse, R. *Nature* **307**, 536–538 (1984).
9. Swainbank, I. G. thesis, Columbia Univ. (1967).
10. Chase, C. G. *Nature* **282**, 464–468 (1979).
11. Busse, F. H. *Geophys. Res. Lett.* **10**, 285–288 (1983).
12. Stille, P., Unruh, D. M. & Tatsumoto, M. *Nature* **304**, 25–29 (1983).
13. Hawkesworth, C. J., Norry, M. J., Roddick, J. C. & Vollmer, R. *Nature* **280**, 28–31 (1979).
14. Dupré, B., Hamelin, B., Allegre, C. J. & Manhès, G. *U.S. geol. Surv. Open File Rep.* 78-701, 103 (1978).
15. Davies, G. F. *Nature* **290**, 208–213 (1981).
16. Allegre, C. J., Brevart, O., Dupré, B. & Minster, J. F. *Phil. Trans. R. Soc.* **297**, 447–477 (1980).
17. Jacobsen, S. B. & Wasserburg, G. J. *J. geophys. Res.* **84**, 7411–7427 (1979).
18. DePaolo, D. J. *Geochim. cosmochim. Acta* **44**, 1185–1196 (1980).
19. O'Nions, R. K., Evensen, N. M. & Hamilton, P. J. *J. geophys. Res.* **84**, 6091–6101 (1979).
20. Armstrong, R. L. *Rev. Geophys.* **6**, 175–199 (1968).
21. Hofmann, A. W. & White, W. M. *Earth planet. Sci. Lett.* **57**, 421–436 (1982).
22. Chase, C. G. *Earth planet. Sci. Lett.* **52**, 277–284 (1981).

The next step is to define the Dupal anomaly more clearly by analysis of basaltic rocks from both oceanic and continental regimes in the Southern Hemisphere. It will also be useful to search for Dupal signatures in Southern Hemisphere volcanic arcs.

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23. Craig, H. & Rison, W. *EOS* **64**, 348–348 (1983).
24. McKenzie, D. & O'Nions, R. K. *Nature* **301**, 229–231 (1983).
25. Sinha, A. K. *Yb Carnegie Instn Wash.* **69**, 405–408 (1971).
26. Cumming, G. L. & Richards, J. R. *Earth planet. Sci. Lett.* **28**, 155–171 (1975).
27. Richards, J. R., Fletcher, I. R. & Blockley, J. G. *Miner. Depos.* **16**, 7–30 (1981).
28. Manhès, G., Allegre, C. J., Dupré, B. & Hamelin, B. *Earth planet. Sci. Lett.* **44**, 91–104 (1979).
29. Allegre, C. J., Dupré, B., Lambret, B. & Richard, P. *Earth planet. Sci. Lett.* **52**, 85–92 (1981).
30. Sun, S. S. *Phil. Trans. R. Soc.* **297**, 409–445 (1980).
31. Taras, B. *EOS* **64**, 907 (1983).
32. Church, S. E. & Tatsumoto, M. *Contr. Miner. Petrol.* **53**, 253–279 (1975).
33. Roden, M. F., Frey, F. A. & Francis, D. M. *J. Petrol.* (in the press).
34. Zartman, R. E. & Tera, F. *Earth planet. Sci. Lett.* **20**, 54–66 (1973).
35. Zindler, G. A. thesis, MIT (1980).
36. Kay, R. W., Sun, S. S. & Lee-Hu, C. N. *Geochim. cosmochim. Acta* **42**, 263–273 (1978).
37. Oversby, V. M. *Geochim. cosmochim. Acta* **36**, 1167–1179 (1972).
38. Sun, S. S. & Hanson, G. N. *Contr. Miner. Petrol.* **52**, 77–106 (1975).
39. Stuckless, J. S. & Ericksen, R. L. *Contr. Miner. Petrol.* **58**, 111–126 (1976).
40. Roden, M. K., Hart, S. R., Frey, F. A. & Melson, W. G. *Contr. Miner. Petrol.* **85**, 376–390 (1984).
41. Oversby, V. M. *Earth planet. Sci. Lett.* **11**, 401–406 (1971).
42. Richardson, S. H., Erlank, A. J., Duncan, A. R. & Reid, D. L. *Earth planet. Sci. Lett.* **59**, 327–342 (1982).
43. Cooper, J. A. & Green, D. H. *Earth planet. Sci. Lett.* **6**, 69–76 (1969).
44. Dasch, E. J. & Green, D. H. *Am. J. Sci.* **275**, 461–469 (1975).
45. Sinha, A. K., Davis, G. L., Hart, S. R. & Krogh, T. *Yb Carnegie Instn Wash.* **69**, 386–388 (1971).
47. Futa, K. & LeMasurier, W. E. *Contr. Miner. Petrol.* **83**, 38–44 (1983).

Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences

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Two related, but distinct, cDNA clones have been isolated and sequenced from a functional murine cytotoxic T-lymphocyte clone. The genes corresponding to these cDNA are expressed and rearranged specifically in T cells and both have similarities to immunoglobulin variable and constant region genes. It is concluded that these genes code for the two subunits of the heterodimeric antigen receptor on the surface of the T cell; its complete deduced primary structure is presented.

THE most characteristic feature of the vertebrate immune system is its capacity to respond to an enormously diverse set of antigenic determinants. At the molecular level this capacity has been traced to an equally diverse set of glycoproteins that are synthesized in B and T lymphocytes. The glycoproteins produced by B cells, called antibodies or immunoglobulins, recognize and bind free antigens and are responsible for humoral immunity, while the T-cell molecules, commonly called T-cell receptors, recognize cell-bound antigens in the specific molecular context of self major histocompatibility complex (MHC) products^{1–3} and are responsible for cellular immunity. The MHC-restriction is an acquired phenomenon^{4,5} determined not by the genotype of the T cells but by that of the host thymus in which the T cells differentiate.

During the past several years extensive studies on the biology and chemistry of immunoglobulin molecules and their genes have illuminated structural and functional properties of these physiologically critical molecules and the genetic origins of their enormous diversity⁶. However, in spite of the equally central

position they occupy in immunological phenomena, the chemical nature of the T-cell receptors has been elusive⁷. Only recently has the first glimpse of these molecules been obtained through the development of effective antisera and monoclonal antibodies that recognize and precipitate clone-specific proteins on the surface of functional T-cell clones, hybridomas or T-cell tumours^{8–10}. These studies suggested that the portion of the receptor determining specificity is a heterodimeric glycoprotein corresponding to a molecular weight (M_r) of about 90,000 (90K) consisting of a 40–45K α -subunit and a 42–44K β -subunit. Moreover, peptide fingerprint analysis suggests that both the α - and β -subunits, like immunoglobulin heavy and light chains, are composed of variable (V) and constant (C) regions^{11–13}. These studies, however, have not provided a primary structure because of the difficulty in preparing a sufficient amount of purified receptor protein.

More recently two groups of workers, using an entirely different approach, succeeded in isolating T-cell-specific cDNA clones of mouse or human origin which show significant

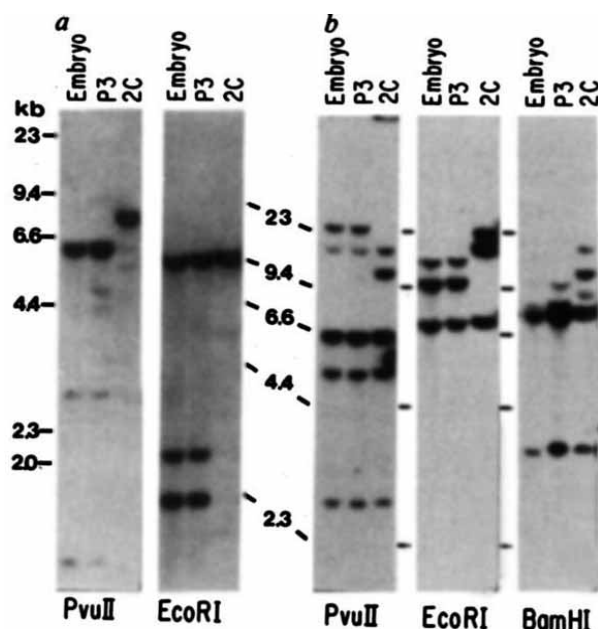


Fig. 1 Southern blot analysis of DNA from BALB/c embryos, myeloma P3 (BALB/c derived), and CTL 2C (BALB.B). DNA was digested with the indicated restriction enzymes, electrophoresed through 0.8% agarose, blotted onto nitrocellulose, and hybridized with 32 P-labelled, nick-translated inserts from clone pHDS11 (a) or clone pHDS4 (b). Hybridization was carried out at 42 °C in 50% formamide and 4 $\times$ SSC. The filters were washed at 65 °C in 0.25% SSC. Separate experiments showed that BALB/c and BALB.B embryo patterns are indistinguishable.

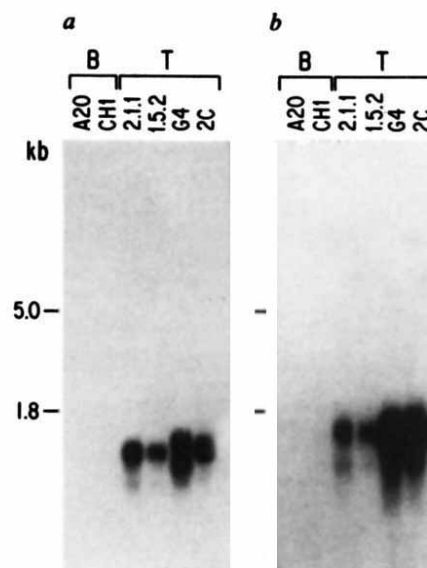


Fig. 2 RNA blot analysis of poly(A)⁺ RNA from various B- and T-cell lines. RNA was extracted from B-cell lymphomas A20-2J and CH1 and alloreactive (H-2^b anti-H-2^d), cytotoxic T-lymphocyte clones 2.1.1, 1.5.2, G4 and 2C. Approximately 1.5 μ g of poly(A)⁺ RNA were denatured with glyoxal and electrophoresed through 1% agarose in 10 mM sodium phosphate buffer, pH 6.5. RNA was transferred to nitrocellulose and hybridized to 32 P-labelled, nick-translated inserts from clone pHDS11 (a) or clone pHDS4 (b). Positions of rRNA markers (5.0 and 1.8 kb) are as indicated.

homology to immunoglobulin genes¹⁴⁻¹⁶. Consistent with their interpretation that these cDNA clones represent one of the two subunits of the receptor, one group has shown that the cDNA encodes a protein composed of an amino-terminal V and a carboxy-terminal C region¹⁶. They also showed that the corresponding genomic DNA sequences have undergone clone-specific somatic rearrangements in various T-cell lines^{15,16}. As the predicted amino acid sequences encoded by the mouse and human cDNA clones isolated by Hedrick *et al.*¹⁶ and Yanagi *et al.*¹⁴, respectively, are highly similar in the C regions, it is very likely that they represent only one of the two subunits of the T-cell receptor.

We have applied the subtraction-cloning method of Davis and his co-workers¹⁵ to a clone of murine alloreactive, cytotoxic T lymphocytes (CTL) and have identified two clearly distinct, mutually non-cross-hybridizing T-cell-specific cDNA clones. Both of these clones show significant sequence homology to immunoglobulin light and heavy chains as well as to the putative receptor genes of Hedrick *et al.*¹⁶ and Yanagi *et al.*¹⁴. In fact, one of our cDNA clones and those of Hedrick *et al.* have a virtually identical nucleotide sequence in the entire constant and 3' untranslated regions. In addition, the genes corresponding to both types of our cDNA have undergone rearrangements in this as well as other cytotoxic T-cell clones.

On the basis of these findings we believe that we have identified and sequenced the genes coding for both subunits of the heterodimeric T-cell receptor of a functional CTL clone. We report here the predicted complete primary structure of this T-cell receptor.

Isolation of T-cell-specific cDNA clones

The alloreactive CTL clone 2C, of BALB.B origin and specific for products of the D end of the BALB/c H-2 complex (d haplotype), has been described previously¹⁷. The cDNA synthesized on the poly(A)⁺ RNA from 2C was subtracted twice with

poly(A)⁺ RNA from a mouse B-cell lymphoma, A20-2J (ref. 18), according to Hedrick *et al.*¹⁵ and a library was constructed from the subtracted cDNA using the vector pBR322 and a standard G-C tailing method. A quarter of this library, containing about 20,000 independent cDNA clones, was subjected to differential screening using two hybridization probes. The first was the 2C cDNA prepared from the poly(A)⁺ RNA of membrane-bound polysomes by subtraction with poly(A)⁺ RNA from another B-cell lymphoma, CH-1 (ref. 19). The second was the cDNA prepared from the total poly(A)⁺ RNA from A20-2J. One hundred and forty 2C-specific cDNA clones were identified and their plasmid DNA was used as hybridization probes in a series of RNA blotting experiments in order to confirm the T-cell-specific expression of the corresponding genes. This permitted us to group the T-cell-specific cDNA clones into at least 10 sets according to the sizes of the corresponding mRNA present in 2C. One was judged to be for the T-cell-specific surface marker Thy-1 on the basis of its hybridization to a previously identified Thy-1 cDNA clone (a gift of Mark Davis).

Two distinct cDNA classes

As was the case in the work of Hedrick *et al.*^{15,16}, one of our assumptions was that the receptor genes in question are rearranged in CTL. Thus we used representative cDNA clones of each set as hybridization probes and compared *Eco*RI-digested genomic DNA from 2C and BALB.B embryos by Southern blot analysis (data not shown). This screening led to the identification of two distinct classes of cDNA, one represented by clone pHDS11 and the other by clones pHDS4 and pHDS203, the genes of which are rearranged in 2C. The other classes of cDNA did not give any indication of DNA rearrangement.

Figure 1a, b shows the results of more extensive Southern blot analysis carried out using clone pHDS11 and pHDS4 as hybridization probes, respectively. The results can be summarized as follows. First, the hybridization patterns obtained by the

two probes are clearly distinct, confirming that the two classes of cDNA clones represent two different sets of genes that do not cross-hybridize to a detectable level in the conditions used. Second, with either probe the hybridization patterns obtained with 2C DNA are different from the patterns obtained with BALB embryo DNA. Third, the myeloma P3 DNA patterns are the same as the embryo DNA patterns. In addition, DNA from five different CTL clones was analysed and each gave patterns different from those of embryo DNA with at least one enzyme (data not shown). Considering the multiplicity and specificity of the pattern differences, these results strongly suggest that genes corresponding to both cDNAs have undergone gross sequence rearrangement in 2C.

The T-cell-specific expression of these genes has been confirmed by analysing poly(A)⁺ RNA from 2C and three other independently derived alloreactive CTL as well as two B-cell lymphomas, A20-2J and CH1. As shown in Fig. 2*a, b*, each of the two cDNA probes detected RNA of distinct sizes in all four CTL but not in either of the B-cell lymphomas, although the relative content, and in some cases the size, of the RNA varied somewhat between CTL. When analysed with the clone pHDS11 probe, CTL 2C, 1.5.2 and 2.1.1 all gave a major RNA component of about 1.3 kilobases (kb) while CTL G4 contained two major components of 1.4 and 1.2 kb. On the other hand, all four CTL gave a 1.5 kb RNA component with the clone pHDS4 probe.

Nucleotide sequence analysis

The restriction maps of the three cDNA clones, pHDS11, pHDS4 and pHDS203, were constructed by standard procedures (Fig. 3), and the DNA sequences were determined by the method of Maxam and Gilbert²⁰ according to the strategy shown in Fig. 3. As both the restriction maps and DNA sequences (see below) confirm that pHDS4 and pHDS203 are derived from the same gene while pHDS11 represents a distinct one, we will describe the sequence features of the two genes separately.

The entire nucleotide sequence of the 1,054 base pair (bp) insert of clone pHDS11 is shown in Fig. 4a. The longest open reading frame is composed of 879 nucleotides whose corresponding amino acid sequence of 293 residues is also shown in Fig. 4a. For reasons given below, the codons are numbered starting with the triplet GAC at nucleotide positions 36–38. Between codons 110 (Glu) and 236 (Cys) the pHDS11 sequence is identical to the sequence of the corresponding region of Hedrick *et al.*'s thymocyte cDNA clone, 86T1 (ref. 16), except for one base pair difference in codon 159 (Arg). According to these authors this region defines the major body of the C region: codons 109 (Leu) and 110 (Glu) define the joining (J) and C region boundary while codon 236 (Cys) precedes the N-terminus of the transmembrane segment. The sequence identity is even more striking between pHDS11 and 2B4.71, the latter being a cDNA clone isolated from a T-helper cell hybridoma specific for pigeon cytochrome c (ref. 21). From codon 109 (Leu) the two sequences are virtually identical throughout the C regions and the entire 3' untranslated regions. The exceptions are two base pair differences, one in codon 159 (Arg) and the other at nucleotide position 992.

Between codons 97 and 109 the pHDS11 sequence is similar to but distinct from the corresponding sequence of 86T1 or 2B4.71. As pointed out by Hedrick *et al.*¹⁶, this region is strikingly homologous to the *J* segments of immunoglobulin genes. In fact the pHDS11 *J* sequence corresponds exactly to the sequence of the *J*_T7 genomic segment recently identified and characterized by Chien *et al.*²¹.

Between codons 1 and 97 the pHDS11 sequence is quite different from the sequence of the corresponding region of either 86T1 or 2B4.71 (Fig. 5*a*). However, patches of conserved residues clearly exist throughout the three sequences and also between these sequences and those encoding immunoglobulin V regions of both light and heavy chains (the latter not shown). Particularly noteworthy conservations are the two Cys residues that are involved in intra-domain disulphide linkages in immunoglobulin V regions (residues 23 and 91 in Figs 4*a* and

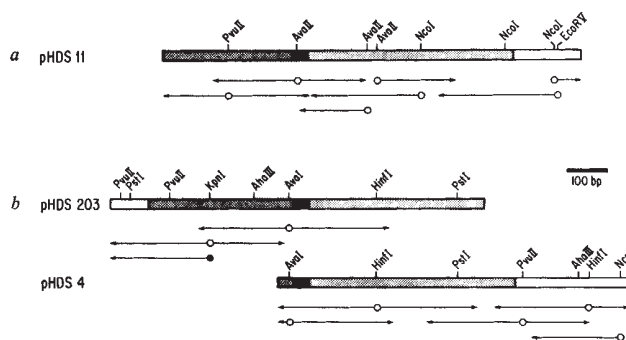


Fig. 3 Restriction maps of the inserts of cDNA clones isolated from CTL 2C. The maps were constructed by the standard single, double or triple digestions of the plasmid DNA. The V, J, C and 5'- or 3'-untranslated regions are shown by , , , respectively. Also shown are the sequencing strategies used to produce the nucleotide sequences shown in Fig. 4. The 5'- and 3'-end labellings are indicated by  and , respectively.

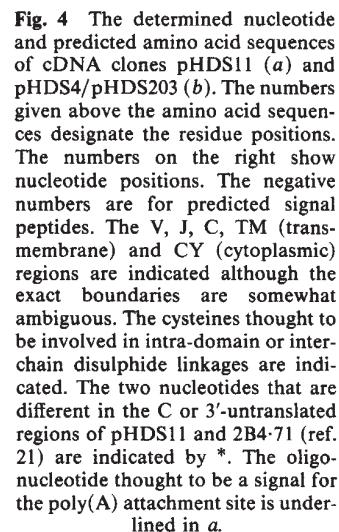
5a). The Trp residue that is universally conserved in immunoglobulin V regions is also present in the corresponding position of the T-cell proteins (residue 34).

At the 5' end of the open reading frame (see Fig. 4a) there is a stretch of 11 highly hydrophobic residues that probably comprise part of a signal peptide²². The position of the N-terminal residue of the mature protein is ambiguous. However, homology between the pHDS11 protein and immunoglobulin V regions, particularly V_κ regions²³, suggests that Asp at position 1 is the N-terminal residue.

As already pointed out by Hedrick *et al.*¹⁶, another stretch of a highly hydrophobic region of about 22 residues, evidently constituting a transmembrane peptide, occurs immediately before the C-terminal 5 hydrophilic residues; the latter are thought to extend into the cytoplasm. Overall, the gene defined by pHDS11 can encode a processed protein of 282 residues with a molecular weight of 32K.

The composite nucleotide sequence of 1,286 bp defined by the two overlapping cDNA clones pHDS4 and pHDS203 is shown in Fig. 4b. The longest open reading frame begins with the Met codon at nucleotide positions 88–90, extends over a stretch of 912 bp, and ends at nucleotide position 999. The predicted amino acid sequence is also shown in Fig. 4b. This sequence is significantly homologous to those of pHDS11, 86T1, 93G7 immunoglobulin heavy chain, MOPC603 immunoglobulin κ light chain, and MOPC104E immunoglobulin λ 1 light chain. This is shown in Figs 5b and 6 where the pHDS4/203 protein sequence is compared with the V (or V+D), J and C region sequences of the other five proteins. Homology is evident in all three regions. The relatedness of the pHDS4/203 protein to the other five proteins in each of the three regions ranges over 18–23% for the V or V+D region, 21–50% for the J region and 16–22% for the C region. The similarity occurs in patches and in the case of the V (or V+D) region these patches tend to correspond to the framework regions (FR) of immunoglobulin V regions. In addition, many of the residues shared by the five non-pHDS4/203 proteins are also shared by the pHDS4/203 protein. These residues (indicated in Fig. 5b by *) include the Cys residues involved in the intra-domain disulphide linkages as well as the Trp residue which is universally present at the boundary of the first hypervariable (HV-1) and FR-2 segments of all immunoglobulin V regions studied to date²³.

As in the pHDS11 protein, the peptide at the amino-terminal end of the pHDS4/203 protein is highly hydrophobic and most probably constitutes a signal peptide. Accordingly the N-terminal residue of the processed protein cannot be unambiguously determined from the cDNA sequence. However, we believe that the N-terminal residue is the Gln at position 1 in Figs 4b and 5b. This is because the N-terminal residue of the α -subunit of the T-cell receptor is blocked, probably by a pyrrolidone carboxylic acid residue (S. Schlossman, personal



The gene defined by cDNA clones pHDS4 and pHDS203 is also expressed and rearranged specifically in T cells. This gene and the β -chain gene are clearly distinct and do not cross-hybridize in standard conditions, although they are related. At the protein level the pHDS4/203 sequence is approximately as closely related (16–23%) to immunoglobulin light and heavy chains as is the β -chain. About the same level (20%) of sequence identity exists between the pHDS4/203 protein and the β -chain. This relatively low overall sequence similarity, however, is somewhat misleading in that it overlooks the striking organizational similarity between the two proteins: each of them contains two

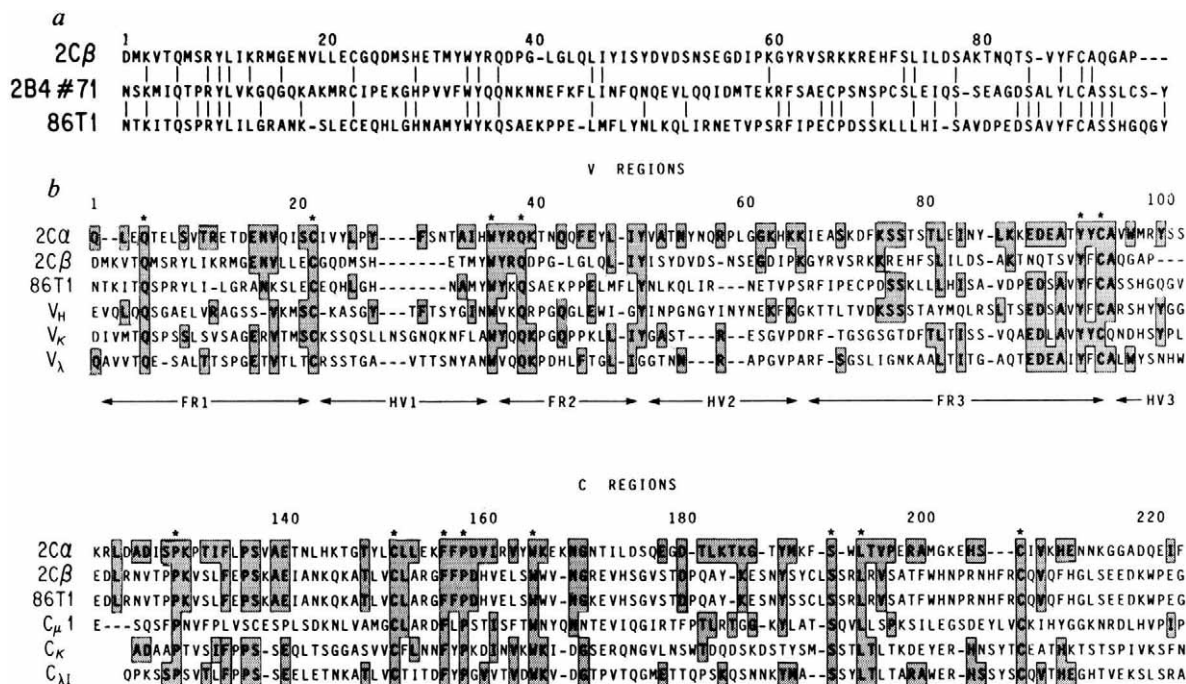


Fig. 5 *a*, Comparisons of the predicted amino acid sequences of three V_β regions, 2Cβ (pHSD11), 2B4.71 (ref. 21) and 86T1 (ref. 16). Those residues common between 2Cβ and 2B4.71, or 2B4.71 and 86T1 are indicated by vertical lines. *b*, Comparisons of the predicted amino acid sequence of pHSD4/203 (2Cα) and predicted or determined amino acid sequences of five other proteins, the β-chain encoded by pHSD11, the β-chain encoded by cDNA clone 86T1 (ref. 16), the V regions of the 93G7 γ1 heavy chain²³, the C_H1 region of the μ heavy chain, the V and C regions of the MOPC603 κ chain²³ and the MOPC104E λ1 chain²³. Those residues common between the 2Cα polypeptide chain and any of the other five chains are shaded. The residues common among all six chains are indicated by *. Approximate boundaries of framework and hypervariable regions as they appear in immunoglobulin V regions are indicated by arrows.

immunoglobulin-like domains, a transmembrane peptide and a cytoplasmic peptide of similar sizes in the two chains. Earlier studies at the protein level indicated that the mouse receptor is composed of two different subunits of similar size held together by one or more disulphide linkage(s)⁸⁻¹⁰. In agreement with these findings, the predicted molecular weight (33K) of the pHSD4/203 protein is very similar to that (32K) of the β-chain encoded by pHSD11. Also, the pHSD4/203 protein and the pHSD11 β-chain each contain one Cys residue in corresponding positions (234 and 236, respectively) that lie outside the immunoglobulin-like domains. These residues can provide the postulated inter-chain disulphide linkage. Thus, while direct evidence is yet to be produced it is very likely that pHSD4/203 codes for the α-subunit of the CTL receptor.

Proposed structure of the T-cell receptor

The amino acid sequences deduced for the two subunits suggest the overall structure for the T-cell receptor shown in Fig. 7. In this model each receptor molecule is made up of two chains, each with two extracellular immunoglobulin-like domains, an amino-terminal variable and a carboxy-terminal constant domain. Each of these domains is stabilized by an S-S bond between cysteine residues that are separated in the linear sequence by 60-70 residues. Amino acid sequence similarity with immunoglobulin V regions (Fig. 5b) point to V (or V+D), D and J segments in the N-terminal domain of the α-subunit (V_α), as previously proposed for the corresponding domain of the β-subunit (V_β)^{16,21}.

Cysteine residues at positions 234 and 236 of the α- and β-subunits, respectively, could well form a single inter-chain S-S bond located close to the cell outer membrane. This bond would link the two subunits in the intact molecule and account for the difference in apparent molecular weight between the unreduced and reduced receptor (90K compared with 40-45K) in SDS-polyacrylamide gel electrophoresis (PAGE). More important, a stable association between two different subunits may well be required to form an effective antigen-binding region,

as is characteristic of immunoglobulins. The obligate participation of two different subunits in the formation of a single combining site means that combinatorial variability is likely to contribute to structural and functional diversity of these receptors.

The protein molecular weights of the α- and β-subunits are each about 10K less than the apparent molecular weight observed in SDS-PAGE. The difference suggests that the subunits are glycosylated. There are four potential sites for N-glycosylation of the β-subunit but none in the α-subunit. It is possible that the α-subunit is O-glycosylated on serine and threonine residues.

After the constant domain, each subunit has at its carboxyl end a hydrophobic stretch of 21-22 amino acids followed by a short stretch of 5(β) or 12(α) amino acid residues in which cationic residues abound. As these segments correspond, respectively, to the transmembrane and cytoplasmic domains that are characteristically found in transmembrane proteins, they support the proposed membrane location and receptor function of the gene products.

The presence of a cysteine residue in the cytoplasmic domain of the α-subunit might provide an SH group for dynamic

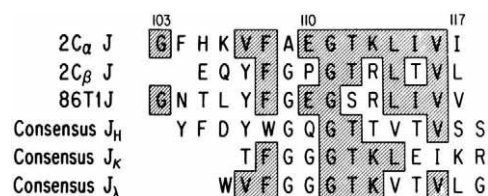


Fig. 6 Comparisons of the J region sequence of 2Cα (predicted from pHSD4/203) and those of 2Cβ (predicted from pHSD11), 86T1 (ref. 16), and immunoglobulin J_H, J_K, and J_λ (ref. 23) consensus sequences. Those residues common between 2Cα J and any one of the other five J segments are boxed in and shaded.

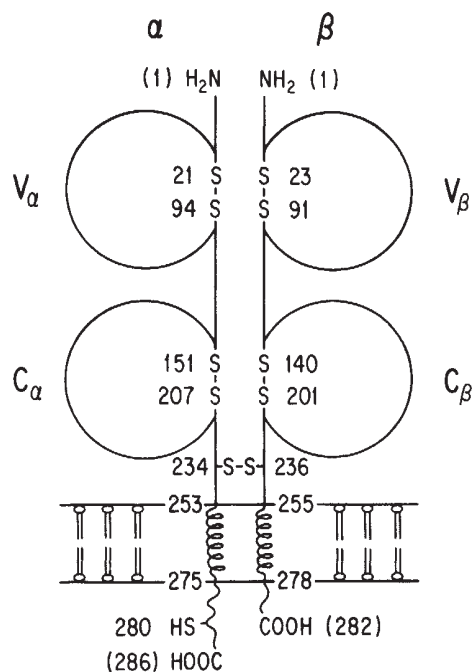


Fig. 7 Proposed overall structure of the 2C receptor. Each receptor molecule is made up of two chains, α and β , each with two extracellular immunoglobulin-like domains, an amino-terminal variable and a carboxy-terminal constant domain. Each of these domains is stabilized by an S-S bond between cysteine residues. Two chains are held by a single inter-chain S-S bond located close to the cell's outer membrane. The protein is anchored on the membrane by two (one for each chain) hydrophobic transmembrane peptides. A short carboxy-terminal peptide rich in cationic residues extends into cytoplasm in each chain. The SH group of the cysteine residue present in the cytoplasmic peptide of the α -subunit may interact dynamically with a membrane or cytoplasmic protein and thereby may be important for the cell's effector function. Both α - and β -chains are glycosylated, although the exact site(s) and extent of glycosylation are unknown.

interactions between the receptor and other molecules of the cell membrane (such as T3 (ref. 9) in human CTL) or perhaps the cytoskeleton. The need for a functional association with such accessory structures is suggested by the overall resemblance of the proposed model to Fab fragments of immunoglobulin molecules: as is well known, these fragments have ligand-binding activity but lack effector functions.

These data throw light on the question of whether CTL and T-helper cell receptors are encoded by the same or separate sets of genes. We will consider the V and C regions separately because the two regions are encoded by distinct gene segments (ref. 21 and our unpublished observations). In immunoglobulin molecules the primary function of the V_H and V_L regions is, of course, to determine antigen specificity. Each of the multiple C_H regions defines an immunoglobulin class and carries a distinct effector function. The primary function of the C_L region is unclear. It is not required for antigen specificity but may be necessary to enhance L-H gene interactions or to facilitate some

unknown function. In T-cell receptors the very presence of the V regions strongly suggests that these regions have a primary role in determining antigen and MHC specificity. An interesting question is whether the CTL and T-helper cell receptors carry distinct C regions each of which has a unique role in the activation or execution of the cell's effector function. An alternative view is that the receptors of the two types of T cells have the same C regions and that while the $\alpha\beta$ heterodimer is specific with regard to ligand binding it has only a nonspecific role in triggering the cell's effector functions.

Comparison of the β cDNA (clone pHDS11) from CTL clone 2C and the β cDNA (clone 2B4.71) from T-helper cell clone 2B4 indicates that at least these CTL and T-helper cells use the same C_β gene segment (clone 2C and clone 2B4 are from the BALB.B and B10.A strains, respectively, and the single base pair differences between their cDNAs may be due to a strain difference.) This conclusion is supported by two observations. First, the near identity of the two cDNA sequences occur not only in the C regions but also in the 3' untranslated regions. Second, the J segments in the two cDNAs are different but belong to the same cluster linked to a single C gene segment²¹. While sequencing of more β genes is required before a firm conclusion can be drawn, these results already suggest strongly that the same C gene segments are used for β chains of CTL and T-helper cells.

Concerning the V regions, several earlier observations suggested that CTL and T-helper cell receptors use non-overlapping sets of gene segments that have evolved separately under different selective pressure: first, the antigen repertoires seem to be quite different; second, different classes of MHC gene products restrict CTL and T-helper cell responses; third, no clearly demonstrated case of T-cell reactivity that crosses the MHC class barrier has been reported (D. Raulet, personal communication). An alternative view is that CTL and T-helper cell receptors share a common pool of germ-line gene segments for V regions and that the distinction between function arises by somatic selection.

The amino acid sequences predicted by the 2C β cDNA (clone pHDS11) and 2B4 β cDNA (clone 2B4.71) are only similar at 21% of residues in the V (or V+D) segment-encoded regions (Fig. 5a). This is substantially lower than the similarity observed between any pair of immunoglobulin V_k segments (40–98%)²³ and is even less than that observed between the C_β region and the immunoglobulin C_k or $C_{\lambda 1}$ domain (22% and 31%, respectively)¹⁶. While characterization of more V_β regions is required before a firm conclusion can be drawn, the weakness of the similarity between the CTL V_β and T-helper cell V_β regions is striking and may reflect independent pools of V and/or D gene segments.

Finally, it will be extremely interesting to compare in similar fashion the V_α and C_α regions of CTL and T-helper cells. It is possible that, as in immunoglobulin molecules, the C region of only one of the two types of chains in these antigen-recognition molecules is involved in effector function.

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- Kindred, B. & Shreffler, D. C. *J. Immun.* **109**, 940–943 (1972).
- Katz, D. H., Hamaoka, T., Dorf, M. E., Maurer, P. H. & Benacerraf, B. *J. exp. Med.* **138**, 734–739 (1973).
- Zinkernagel, R. M. & Doherty, P. C. *Nature* **248**, 701–702 (1974).
- Zinkernagel, R. M. *et al. J. exp. Med.* **147**, 882–896 (1978).
- Fink, P. J. & Bevan, M. J. *J. exp. Med.* **148**, 766–775 (1978).
- Tonegawa, S. *Nature* **302**, 575–581 (1983).
- Jensenius, J. C. & Williams, A. F. *Nature* **300**, 583–588 (1982).
- Allison, J. P., McIntyre, B. W. & Bloch, D. J. *Immun.* **129**, 2293–2300 (1982).
- Meur, S. C. *et al. J. exp. Med.* **157**, 705–719 (1983).
- Haskins, K. *et al. J. exp. Med.* **157**, 1149–1169 (1983).
- Acuto, O. *et al. Cell* **34**, 717–726 (1983).
- McIntyre, B. W. & Allison, J. P. *Cell* **34**, 739–746 (1983).

- Kappler, J. *et al. Cell* **35**, 295–302 (1983).
- Yanagi, Y. *et al. Nature* **308**, 145–149 (1984).
- Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. *Nature* **308**, 149–153 (1984).
- Hedrick, S. M., Nielsen, E. A., Kavalier, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153–158 (1984).
- Kranz, D. M., Sherman, D. H., Sitkovsky, M. V., Pasternack, M. S. & Eisen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **81**, 573–577 (1984).
- McKean, D. J. *et al. J. exp. Med.* **154**, 1419–1431 (1981).
- Lynes, M. A., Lanier, L. L., Babcock, G. F., Wettstein, P. J. & Haughton, G. J. *Immun.* **121**, 2352–2357 (1978).
- Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).
- Chien, Yih., Gascoigne, N. R. J., Kavalier, J., Lee, N. E. & Davis, M. M. *Nature* **309**, 322–326 (1984).
- Blöbel, G. & Dobberstein, B. *J. Cell Biol.* **67**, 834–851 (1975).
- Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M. & Perry, H. in *Proteins of Immunological Interest* (National Institutes of Health, Bethesda, 1983).

Molecular cloning of cDNA encoding a murine haematopoietic growth regulator, granulocyte-macrophage colony stimulating factor

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cDNA clones specifying the murine granulocyte-macrophage colony stimulating factor have been isolated. This haematopoietic growth factor is encoded by a unique gene specifying a messenger RNA of 1,200 nucleotides and a polypeptide of 118 amino acids. It bears no structural similarity to the functionally related factor, interleukin-3, described recently.

INDIVIDUAL multipotential stem cells can give rise to all of the different cell types of the haematopoietic and lymphoid systems¹. Although this process is poorly understood, it has become apparent from *in vitro* studies that the proliferation, differentiation and functional activation of the various blood cells are controlled by a group of glycoprotein regulator factors including erythropoietin, T-cell growth factor (IL2) and the colony stimulating factors (CSFs)². The colony stimulating factors which regulate the formation of neutrophilic granulocytes and macrophages have distinctive biological activities (M-CSF is a selective proliferative stimulus for macrophages³; G-CSF for granulocytes⁴; GM-CSF for both granulocytes and macrophages⁵), while multi-CSF—also known as interleukin-3 (ref. 6), burst-promoting activity⁷, P-cell stimulating factor⁸, mast cell growth factor⁹ and haematopoietic cell growth factor¹⁰—stimulates the proliferation of not only neutrophilic granulocytes and macrophages, but also eosinophils, megakaryocytes, erythroid and mast cells¹¹. Although it has been possible to purify all four colony stimulating factors, detailed analysis of many aspects of their biology and biochemistry has been hampered by the limited quantities available. This problem can be largely circumvented by molecular cloning of the corresponding gene sequences.

The present report describes molecular clones corresponding to GM-CSF. GM-CSF synthesized by mouse lung tissue is a glycoprotein of molecular weight 23,000 (ref. 5) which is active at low concentrations (10^{-11} – 10^{-12} M) and is required continuously for the *in vitro* proliferation of progenitor cells of granulocytes and macrophages². It also controls the irreversible commitment of these progenitors to the formation of granulocytes and macrophages¹² and can regulate the functional activity of the mature end cells². A partial NH₂-terminal amino acid sequence has recently been determined for GM-CSF isolated from mouse lung-conditioned medium (L. Sparrow, D.M., T. Hunkapillar, L. Hood and A.W.B., manuscript in preparation). Using this partial amino acid sequence, oligonucleotides complementary to the putative GM-CSF mRNA were synthesized and used as probes to identify cDNA clones corresponding to the GM-CSF messenger RNA in a mouse lung cDNA library. From the nucleotide sequence of the cDNA clones, we deduced the amino acid sequence of GM-CSF and compared it with that recently reported for a functionally related growth factor, multi-CSF (interleukin-3)^{13,14}.

Isolation of GM-CSF clones

All mouse tissues contain and synthesize detectable amounts of GM-CSF, although the levels are extremely low¹⁵. To isolate a GM-CSF complementary DNA clone, we constructed a cDNA library using mRNA from the most active tissue available: lungs from mice injected with bacterial endotoxin¹⁶. The library was

searched for GM-CSF clones by hybridization with synthetic oligonucleotides complementary to two short regions of the putative GM-CSF mRNA sequence, predicted from the partial NH₂-terminal amino acid sequence of the protein. We used oligonucleotide probes complementary to two different regions of the mRNA to help rule out irrelevant clones: whilst some clones may contain sequences fortuitously complementary to one of the oligonucleotide probes, a clone containing the GM-CSF sequence would be expected to hybridize to both. Figure 1 shows the GM-CSF amino acid sequence between residues 7 and 16, the possible combinations of nucleotide sequences that could encode this peptide and the oligonucleotides that were used as hybridization probes. As the assignment of amino acid 9 was equivocal (being either histidine or cysteine), two different sets of oligonucleotides encompassing this region were synthesized; probe 1 assumes a histidine and probe 2 a cysteine residue. The second region chosen within the amino acid sequence required an extremely degenerate set of oligonucleotides which were synthesized as two 48-fold degenerate sets (probes 3 and 4).

In the initial screening, all four sets of oligonucleotide probes were used as a mixture (see legend to Fig. 1); screening of ~100,000 cDNA clones yielded 22 positives, of which two (pGM37 and pGM38) hybridized separately with probe 1 and with a mixture of 3 plus 4 and were therefore strong candidates to contain sequences coding for GM-CSF. In order to confirm their identity, we asked whether transcripts complementary to these cloned sequences were present in cells that synthesize GM-CSF; whether DNA from these clones could specifically select, by hybridization, biologically active GM-CSF mRNA; and whether the nucleotide sequence of the clones could encode the known GM-CSF protein sequence.

Detection of GM-CSF mRNA

Initially we determined whether the abundance of transcripts corresponding to pGM38 in various cell types paralleled the ability of those cells to synthesize GM-CSF. Messenger RNA from mouse lung (which synthesizes GM-CSF) and from various cells which do not synthesize GM-CSF was fractionated on formaldehyde-agarose gels, transferred to nitrocellulose and hybridized with a probe derived from pGM38. Figure 2 shows that this probe detected a low abundance transcript of about 1.2 kilobases (kb) in mRNA from mouse lung (lane 3), but not in mRNA from several randomly selected cell lines which do not synthesize GM-CSF, including the thymoma-derived cell line TIKAUT (lane 1) and the plasmacytoma P3 (lane 2). Furthermore, this probe failed to detect any transcript in mRNA from WEHI-3B D⁻ (lane 6), RIII (lane 7) and L cells (lane 8), which synthesize multi-CSF⁶, G-CSF (N.A.N., G. R. Johnson and R. H. Whitehead, unpublished results) and M-CSF³ respectively. The very low abundance of the transcript corresponding

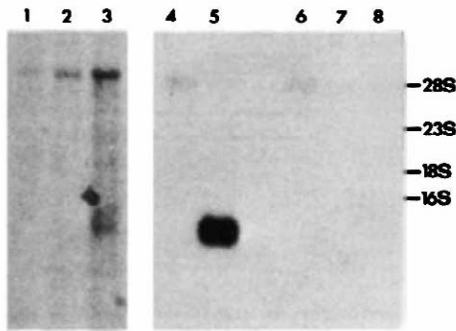


Fig. 2 Detection of GM-CSF mRNA in various cells. 5 μ g of poly(A) RNA from TIKAUT (lane 1), P3 (lane 2), mouse lung (lane 3), unstimulated LB3 (lane 4), concanavalin A-stimulated LB3 (lane 5), WEHI3B D⁻ (lane 6), RIII (lane 7) and L cells (lane 8) were electrophoresed on 1% formaldehyde/agarose gels, transferred to nitrocellulose and hybridized with a fragment of DNA spanning the entire insert of pGM38 ³²P-labelled by nick-translation⁴⁸. Hybridization was at 65 °C for 16 h in 2 $\times$ SSC, 0.1% SDS, 0.2% Ficoll, 0.2% PVP, 0.2% BSA containing 50 μ g ml⁻¹ of denatured salmon sperm DNA. LB3 (formerly B3)²⁴ is a cloned Thy-1⁺, Lyt-2⁻, MT4⁺ T lymphocyte line derived from BALB/c anti-DBA/2 mixed leukocyte culture, and maintained by weekly passage with irradiated DBA/2 spleen cells (1,500 R) in the presence of interleukin-2. Concanavalin A-stimulated LB3 cells were prepared by culturing cells at 10⁶ per ml with 5 μ g ml⁻¹ concanavalin A in tissue culture medium with 5% fetal calf serum for 5 h. The molecular weight markers used and their presumed molecular weights were: mammalian 28 and 18S rRNA (4,700 and 1,800 nucleotides) and *E. coli* 23 and 16S rRNA (2,904 and 1,541 nucleotides). A low level of hybridization to residual 28S rRNA is evident after long autoradiographic exposure (tracks 1–3).

the mRNA is shown in Fig. 5a. The nucleotide sequence given in Fig. 5b is a composite derived from both clones. A stretch of 20 adenosine residues, corresponding to the poly(A) tail of the mRNA, preceded by the hexanucleotide AATAAA (found toward the 3' terminus of most eukaryotic mRNAs²³) is present at one end of pGM38. This allowed us to orient the sequences of the cDNA clones with that of the mRNA.

The sequence presented in Fig. 5b contains a single large open reading frame of 354 nucleotides; the amino acid sequence predicted by this region is presented from the first residue of the mature protein and is given above the nucleotide sequence. From residues 2 to 29, the amino acid sequence is identical (but for four residues) with the partial NH₂-terminal amino acid sequence for GM-CSF (L. Sparrow *et al.*, manuscript in preparation), which is shown above that predicted by the cDNA. Of the four discrepancies, two of the residues (20 and 24) had been only tentatively assigned in the sequence of the protein. The first residue of the mature peptide, which could not be determined in the protein sequence, is predicted by the nucleotide sequence to be isoleucine. The primary structure of the protein deduced from the nucleotide sequence has a molecular weight of 13,500, which is in good agreement with the apparent molecular weight of 16,800 for GM-CSF extensively deglycosylated with endoglycosidase F (A.W.B., unpublished results). Two potential *N*-glycosylation sites (Asn-X-Thr)²⁴ which occur within the predicted amino acid sequence, are indicated in Fig. 5b by asterisks.

pGM37 extends 20 nucleotides 5' to the first position presented in Fig. 5b and does not contain an initiation codon; several hydrophobic amino acids are contained within this region (not shown), a characteristic of signal peptides of secreted proteins²⁵. The size of the GM-CSF mRNA is ~1,200 nucleotides (Fig. 2), of which ~150 nucleotides are probably contributed by the poly(A) tail²⁶. The 3' untranslated region is 319 nucleotides and the region encoding the mature protein 354 nucleotides (Fig. 5b). Thus some 350 nucleotides remain for the putative NH₂-terminal signal peptide and the 5' untranslated region.

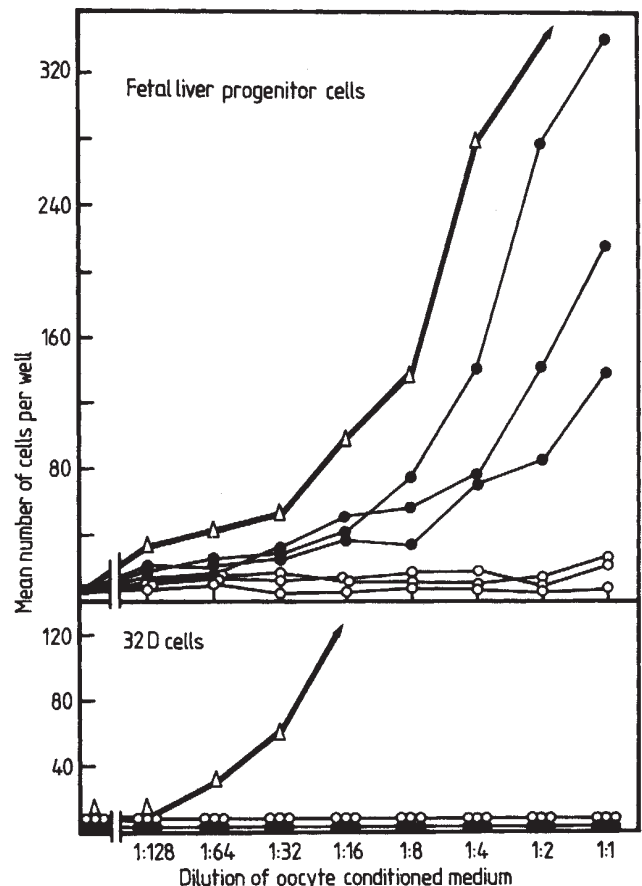


Fig. 3 Stimulation of cellular proliferation in suspension cultures of haematopoietic progenitor cells from fetal liver (upper panel) and multi-CSF-dependent 32D C13 cells (lower panel) by medium from mRNA-injected *Xenopus* oocytes. Unfractionated LB3 mRNA is indicated by heavy lines. ●—●, mRNA selected by hybridization to pGM38 DNA; ○—○, that selected by vector DNA alone; the three separate curves represent triplicate experiments. **Methods:** Hybrid selection was performed essentially as described elsewhere⁴⁹. pGM38 or vector (pJL3) DNA (5- μ g aliquots) were bound to small squares of nitrocellulose and incubated with 1–2 μ g aliquots of concanavalin A-stimulated LB3 mRNA in 50% formamide, 0.5 M Tris-HCl pH 7.5, 0.75 M NaCl, 0.002 M EDTA, 0.4% SDS, 10 μ g ml⁻¹ *E. coli* tRNA in a reaction volume of 30 μ l at 37 °C for 16 h. After incubation, filters were exhaustively washed in hybridization buffer at 37 °C and then in 10 mM Tris-HCl pH 7.5, 2 mM EDTA at 52 °C. Bound RNA was eluted by boiling for 1 min in 300 μ l 10 mM Tris-HCl pH 7.5, 2 mM EDTA containing 1.5 μ g of *E. coli* tRNA. RNA was precipitated by addition of sodium acetate and ethanol and chilling at -20 °C for 16 h. After centrifugation, precipitated RNA was washed with cold 70% ethanol, dried and redissolved in 1.5 μ l 1 mM Tris-HCl pH 7.5, 0.1 mM EDTA. For each RNA sample, groups of 30 *Xenopus* oocytes were injected with 50 nl of RNA per egg. Unfractionated LB3 mRNA was at 1 μ g ml⁻¹ for injection and 50 nl was injected per egg. After incubation of injected oocytes for 4 days, oocyte culture medium was diluted 1:2 in medium containing 5% fetal calf serum, filtered and 5- μ l volumes assayed in serial dilutions in 15- μ l cultures containing 200 fluorescence-activated cell sorter fractionated 14 day CBA fetal liver progenitor cells¹⁸ or 200 32D C13 cells²⁰. Each point is the mean cell count from duplicate cultures after 2 days of incubation.

There are three discrepancies between the nucleotide sequences derived from the two cDNA clones (positions 237, 346 and 507), one of which (position 346) causes an amino acid sequence ambiguity (Gly or Ser at amino acid residue 116). As the mice (C57BL/6) from which the cDNA clones were isolated are highly inbred and hence should be homozygous at the GM-CSF locus, and as there appears to be only one gene encoding GM-CSF in the mouse germ line (see below), it is

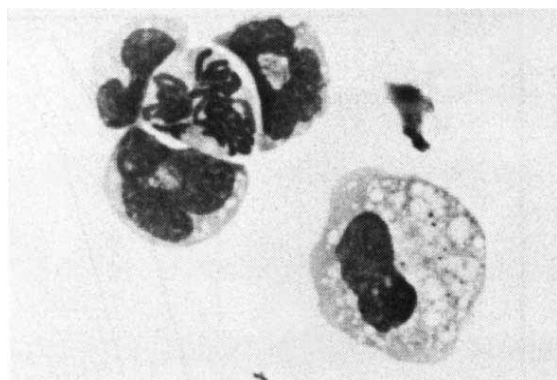


Fig. 4 Four-day suspension culture of purified fetal liver haematopoietic progenitor cells after stimulation by medium from oocytes injected with pGM38-selected mRNA from LB3 cells. Note mitotic activity and the production of maturing granulocytes and macrophages.

likely that these three sequence ambiguities reflect artefacts created during cDNA synthesis²⁷, and indeed reverse transcriptase has an error frequency on synthetic polynucleotides of approximately one in 600 nucleotides (ref. 28).

GM-CSF is encoded by a unique gene

As there are known to be multiple molecular forms of GM-CSF isolated from various mouse organs²⁹, we tested how many genes the GM-CSF cDNA could detect in the murine genome. BALB/c embryo or C57BL/6 liver DNA digested with various restriction endonucleases was fractionated on agarose gels, transferred to nitrocellulose³⁰ and hybridized with a fragment containing the entire cDNA insert in pGM38. In both mouse strains, a single *Eco*RI fragment (Fig. 6, lanes 1, 2), a single *Pst*I fragment (lanes 3, 4) and a single *Bam*HI fragment (not shown) were detected. Since the *Pst*I fragment is only 2.5 kb in length it is unlikely that this fragment could accommodate more than one gene. We therefore conclude that there is only one gene encoding the lung-type GM-CSF in the mouse germ line, barring the unlikely possibility that two (or more) genes are flanked by identically positioned *Eco*RI, *Pst*I and *Bam*HI restriction sites.

Figure 6 also reveals that the GM-CSF gene is contained within the same *Eco*RI fragment in DNA from concanavalin A-stimulated LB3 cells (lane 6) as in embryo DNA (lane 5). No additional bands were evident in LB3 DNA nor was the intensity of hybridization different from that of embryo DNA, from which we infer that there are no alterations in the copy number or gross changes in the context of the GM-CSF gene in cells which synthesize large quantities of GM-CSF.

In order to determine the chromosome on which the GM-CSF gene resides, we examined DNA from seven mouse-Chinese hamster hybrid cell lines which retain various mouse chromosomes (see Table 1 in ref. 31). Southern analysis of the cell lines (not shown) indicated that the 22 kb *Eco*RI fragment present in the murine genome was absent from every hybrid cell line. As chromosome 11 is the only mouse chromosome not retained in any of these lines³¹ (a characteristic of mouse-Chinese hamster hybrids³²), we conclude that the GM-CSF gene is likely to be located on this chromosome. The GM-CSF gene is therefore unlikely to be linked to the four loci in the mouse, *Sl*, *W*, *an* (ref. 33) and *op* (ref. 34), which are involved in the control of haematopoietic stem cell development and which are located on chromosomes 10, 5, 4 (ref. 33) and 12 (ref. 35) respectively.

Comparison with other growth factors

Four distinct colony stimulating factors with distinguishable biochemical and biological properties have been found to act within the granulocyte-macrophage pathway. Furthermore, multiple molecular forms of each of these factors have been observed^{16,35-37}: the GM-CSF extracted from various mouse organs have different physical properties¹⁶; indeed, it has been

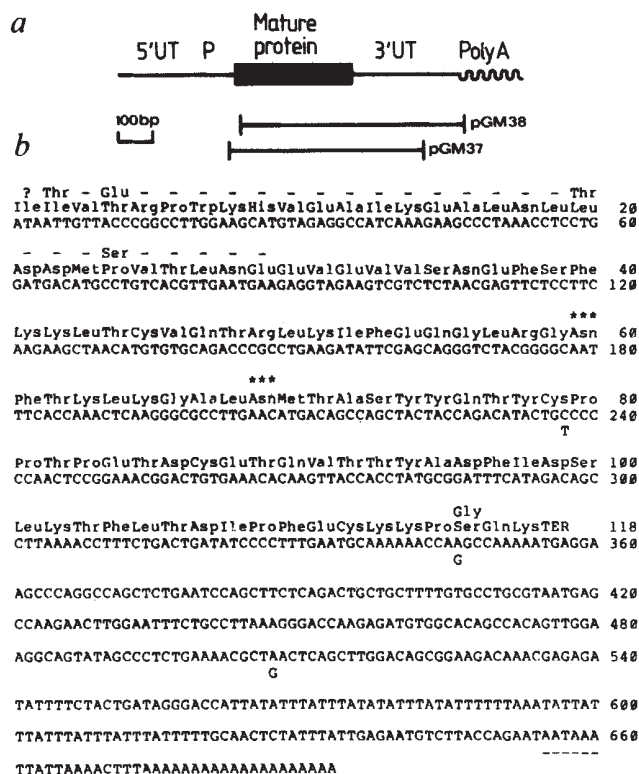


Fig. 5 *a*, Map of the GM-CSF mRNA and of clones pGM37 and pGM38. The mRNA is taken to be 1,200 nucleotides in length. The region of the mRNA encoding the mature protein is shown as a thick line. The untranslated regions are designated by UT and a putative precursor peptide by P. The regions contained within clones pGM37 and pGM38 are indicated with bars. pGM38 extends from nucleotide 14 in the sequence presented in *b* to the poly(A) tail, whereas pGM37 extends from 20 nucleotides 5' to the sequence presented to position 574. *b*, Nucleotide sequence of GM-CSF mRNA. DNA fragments subcloned in M13 vectors⁵⁰ were sequenced by the chain-termination method using dideoxynucleoside triphosphates⁵¹. Sequencing reactions were electrophoresed on thermostatically-controlled, 0.2 mm thick, 8% (w:v) polyacrylamide gels⁵². The nucleotide sequence given is a composite of sequence derived from clones pGM37 and pGM38; at three positions where the pGM37 nucleotide sequence differs from that of pGM38 the pGM37 alternatives are given below the line. The sequence of the mRNA-synonymous strand is listed 5' to 3', with the predicted amino acid sequence of GM-CSF given above; numbers at the ends of lines indicate the position of the final residue (amino acid or nucleotide) on that line. The partial amino acid sequence determined by L. Sparrow *et al.* (manuscript in preparation) for GM-CSF is indicated above the sequence derived from the clones; at positions where there is no discordance between the two, dashes are given. The first amino acid residue, determined by analysis of the protein, could not be assigned, and is indicated by a question mark. Potential *N*-glycosylation sites²⁴ are indicated with asterisks, and the putative polyadenylation signal²³ is underlined.

suggested that the GM-CSF produced by T lymphocytes may also represent a distinct form³⁸. However, after appropriate treatment to reduce carbohydrate and charge heterogeneity, the GM-CSF from different organs have been shown to have nearly identical biological and biophysical properties¹⁵. Nonetheless, it is still unclear whether they represent products of multiple related genes or a single gene product with post-translational modifications. However, as we have shown here that there is only one gene in the mouse germ line corresponding to the lung-type GM-CSF, it is highly likely that the differences in the GM-CSF produced by different adult organs reflect post-translational modifications of a single product. Moreover, since the lung-derived GM-CSF cDNA can hybridize to the GM-CSF mRNA in the T-cell line LB3, this T-cell derived GM-CSF must be

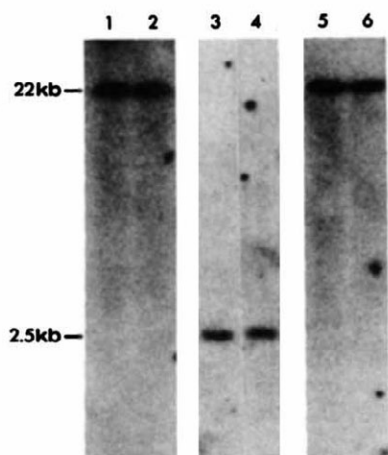


Fig. 6 Detection of the GM-CSF gene. DNA (20 μ g) was digested with *Eco*RI (lanes 1, 2, 5, 6) or *Pst*I (lanes 3, 4) and electrophoresed on 0.8% agarose gels. After transfer to nitrocellulose²⁹, the filters were hybridized with a fragment spanning the entire cDNA insert of pGM38, ³²P-labelled by nick-translation²³. Hybridization was at 65 °C for 16 h in 2×SSC, 0.1% SDS, 0.2% Ficoll, 0.2% PVP, 0.2% BSA containing 50 μ g ml⁻¹ of denatured salmon sperm DNA. The DNA used in lanes 1, 3 and 5 was from 10 day old BALB/c mouse embryos, that in lanes 2 and 4 from C57BL/6 livers and that in track 6 from concanavalin A-stimulated LB3 cells (see legend to Fig. 2). Fragment sizes are given in kilobases and were determined³³ by reference to a *Hind*III digest of bacteriophage λ DNA.

encoded by the same gene as the lung-type GM-CSF. Variants with different amino acid sequences from that of the lung-type GM-CSF, and whose genes do not cross-hybridize with the lung-type GM-CSF, may of course exist.

It is intriguing to compare the sequence of GM-CSF with that of multi-CSF which, in addition to its other activities, has similar actions to GM-CSF in stimulating the growth of granulocyte and macrophage colonies from committed progenitor cells^{11,35}. It might not have been surprising if these two factors had shown primary amino acid sequence similarity, reflecting a common receptor binding site or similar modes of action within the cell. This is, however, not the case. When we compared the amino acid sequence of GM-CSF (Fig. 5b) with that for murine multi-CSF^{13,14} using the ALIGN computer program³⁹, we found only one cluster of three consecutive amino acids in common between

GM-CSF and multi-CSF: Thr-Leu-Asn at position 26–28 of GM-CSF and 40–42 of multi-CSF. The dissimilarity of these two regulators is emphasized further by the differences in the predicted secondary⁴⁰ and tertiary⁴¹ structures. Whereas GM-CSF is predicted to contain two long α -helical segments towards the NH₂-terminus of the molecule (from residues 7–22 and 25–40), the only notable α -helix predicted in multi-CSF is towards the C-terminus (residues 123–132). Furthermore, the hydrophilicity profiles for the two molecules are quite different, suggesting that their surface structures bear little resemblance to one another. Thus it would appear that GM-CSF and multi-CSF represent an example where similar biological activities have arisen by convergent evolution.

The apparent structural dissimilarity of GM-CSF and multi-CSF suggests that they may have different receptor binding sites; indeed, the simplest model would be that these binding sites represent two independent receptors. In this model, a cell responsive to both GM-CSF and multi-CSF would have both receptors, either of which when triggered would stimulate proliferation and differentiation, whereas a cell responsive only to multi-CSF would have a receptor only for this factor. However, this model is complicated by the observation that multi-CSF can compete efficiently with GM-CSF for binding to its receptor on bone marrow cells (F. Walker and A.W.B., unpublished results), suggesting that GM-CSF and multi-CSF may in fact interact with the same receptor.

As the molecular cloning of the murine GM-CSF mRNA sequence should facilitate the production of larger quantities of this regulator molecule than previously available, it should be possible to study in detail the interaction of GM-CSF with its receptor(s) and the signals thus generated. Moreover, by *in vitro* mutagenesis it should be possible to dissect the active site(s) of the molecule and to ask whether the different activities of GM-CSF—survival, proliferation and differentiation of progenitor cells—are determined by the same or different active sites.

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1. Metcalf, D. & Moore, M. A. S. *Haemopoietic Cells* (North-Holland, Amsterdam, 1971).
2. Metcalf, D. in *Tissue Growth Factors* (ed. Baserga, R.) 343–384 (Springer, New York, 1981).
3. Stanley, E. R. & Heard, P. M. *J. biol. Chem.* **252**, 4305–4312 (1977).
4. Nicola, N. A., Metcalf, D., Matsumoto, M. & Johnson, G. R. *J. biol. Chem.* **258**, 9017–9021 (1983).
5. Burgess, A. W., Camakaris, J. & Metcalf, D. *J. biol. Chem.* **252**, 1998–2003 (1977).
6. Ihle, J. N., et al. *J. Immun.* **129**, 2431–2436 (1982).
7. Iscove, N. N., Roitsch, C. A., Williams, N. & Guilbert, L. J. *J. cell Physiol. Suppl.* **1**, 65–78 (1982).
8. Clark-Lewis, I. & Schrader, J. W. *J. Immun.* **127**, 1941–1947 (1981).
9. Yung, Y.-P., Eger, R., Tertan, G. & Moore, M. A. S. *J. Immun.* **127**, 794–799 (1981).
10. Bazill, G. W., Haynes, M., Garland, J. & Dexter, T. M. *Biochem. J.* **210**, 747–759 (1983).
11. Metcalf, D. in *Normal and Neoplastic Haemopoiesis* (eds Golde, D. W. & Marks, P. A.) 141–156 (Liss, New York, 1983).
12. Metcalf, D. & Burgess, A. W. *J. cell. Physiol.* **111**, 275–283 (1982).
13. Fung, M. C., et al. *Nature* **307**, 233–237 (1984).
14. Yokota, T., et al. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1070–1074 (1984).
15. Nicola, N. A., Burgess, A. W. & Metcalf, D. *J. biol. Chem.* **254**, 5290–5299 (1979).
16. Sheridan, J. W. & Metcalf, D. *J. cell. Physiol.* **81**, 11–24 (1973).
17. Kelso, A., MacDonald, H. R., Smith, K. A., Cerottini, J.-C. & Brunner, K. T. *J. Immun.* (in the press).
18. Burgess, A. W., Nicola, N. A., Johnson, G. R. & Nice, E. C. *Blood* **60**, 1219–1223 (1982).
19. Nicola, N. A. & Metcalf, D. *J. cell. Physiol.* **112**, 257–264 (1982).
20. Greenberger, J. S., et al. in *Experimental Hematology Today 1982* (eds Baum, S. J., Ledney, G. D. & Thierfelder, S.) 195–209 (Karger, Basel, 1982).
21. Metcalf, D. *Blood* (in the press).
22. Metcalf, D. *Haemopoietic Colonies* (Springer, Heidelberg, 1977).
23. Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211–214 (1976).
24. Neuberger, A., Gottschalk, A., Marshall, R. D. & Spiro, R. G. in *Glycoproteins* (ed. Gottschalk, A.) 450–490 (Elsevier, Amsterdam, 1972).
25. Von Heijne, G. *Eur. J. Biochem.* **133**, 17–21 (1983).
26. Jeffery, W. R. & Brawerman, G. *Biochemistry* **13**, 4633–4637 (1974).
27. Gough, N. M., Webb, E. A., Cory, S. & Adams, J. M. *Biochemistry* **19**, 2702–2710 (1980).
28. Battula, N. & Loeb, L. A. *J. biol. Chem.* **250**, 4405–4409 (1975).
29. Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
30. Cory, S., et al. *EMBO J.* **2**, 213–216 (1983).
31. Francke, U., et al. *Cytogenet. cell. Genet.* **19**, 57–84 (1977).
32. Russell, E. S. *Adv. Genet.* **20**, 357–459 (1979).
33. Wiktor-Jedrzejczak, W., Ahmed, A., Szczylak, C. & Skelly, R. R. *J. exp. Med.* **156**, 1516–1527 (1982).
34. Marks, S. C. Jr & Lane, P. W. *J. Hered.* **67**, 11–18 (1976).
35. Burgess, A. W., Metcalf, D., Russell, S. H. M. & Nicola, N. A. *Biochem. J.* **185**, 301–314 (1980).
36. Stanley, E. R. & Guilbert, L. J. *J. Immun. Meth.* **42**, 253–284 (1981).
37. Nicola, N. A., Matsumoto, M., Metcalf, D. & Johnson, G. R. in *Modern Trends in Human Leukaemia* Vol. 5 (eds Neth, R., Gallo, R. C., Greaves, M., Moore, M. A. S. & Winkler, K.) 345–347 (Springer, Berlin, 1983).
38. Clark-Lewis, I. & Schrader, J. W. *J. Immun.* **128**, 175–180 (1982).
39. Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 2 (ed. Dayhoff, M. O.) 1–8 (National Biomedical Research Foundation, Washington DC, 1976).
40. Garnier, J., Osguthorpe, D. J. & Robson, B. *J. molec. Biol.* **120**, 97–120 (1978).
41. Hopp, T. P. & Woods, K. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3824–3828 (1981).
42. Gonda, T. J., Sheiness, D. K. & Bishop, J. M. *Molec. cell. Biol.* **2**, 617–624 (1982).
43. Efstratiadis, A., Kafatos, F. C., Maxam, A. M. & Maniatis, T. *Cell* **7**, 279–288 (1976).
44. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
45. Michelson, A. M. & Orkin, S. H. *J. biol. Chem.* **257**, 14773–14782 (1982).
46. Casadaban, M. J. & Cohen, S. N. *J. molec. Biol.* **138**, 179–207 (1980).
47. Hanahan, D. & Meselson, M. *Gene* **10**, 63–67 (1980).
48. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
49. Miller, J. S., Paterson, B. M., Ricciardi, R. P., Cohen, L. & Roberts, B. E. *Meth. Enzym.* **101**, 650–674 (1983).
50. Messing, J. & Vieira, J. *Gene* **19**, 269–276 (1982).
51. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
52. Garoff, H. & Ansorge, W. *Analyt. Biochem.* **115**, 450–457 (1981).
53. Gough, E. J. & Gough, N. M. *Nucleic Acids Res.* **12**, 845–853 (1984).

Red component activity in dwarf novae

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SU Ursae Majoris systems are short-period binary dwarf novae characterized by two distinct classes of outbursts: 'normal outbursts' of duration ~ 2 days and less frequent 'superoutbursts' which last ~ 12 days. Superoutbursts may be present in longer-period systems but could be classified as wide outbursts¹. Superoutbursts require enhanced mass transfer rates for an extended period of ~ 10 days to explain their sustained activity^{2,3}. During superoutbursts, 'superhumps' are observed⁴⁻⁶ which modulate the visible light by $\leq 40\%$ with a period 3–7% longer than the binary period. Measurement of eclipse depths in the two eclipsing SU UMa systems during superoutburst should show whether or not the superhump light is eclipsed and hence make it possible to infer its location either in the eclipsed disk or in the uneclipsed red component. Study of the available eclipse data reported here shows that the superhump light is not eclipsed, so that the red component is the location of the superhump.

Several models^{4,7,8} locate the superhump in the vicinity of the accretion disk. Hence the superhump is at least partially eclipsed at the superhump maximum. Furthermore, models based on eccentric structure of the outer disk have significant difficulties on dynamical grounds^{9,10}. The red-component superhump model due originally to Warner⁵ and also suggested by Vogt⁶ and Bath *et al.*¹¹ places the superhump on the red star and it would not be eclipsed (Fig. 1).

The eclipse acts as a 'stroboscope', revealing the brightness of the non-eclipsed material at various phases of the superhump light. In models where the superhump light is eclipsed the brightness at mid-eclipse will be unchanged by the passage through superhump maximum. A non-eclipsed superhump would cause the eclipsed light level to rise simultaneously with the out-of-eclipse superhump light¹¹. The profile of the non-eclipsed light against superhump phase can be mapped using data from several eclipses.

The data analysed are those of Warner⁴, and Krzeminski (see ref. 12). We have examined data for Z Chamaeleontis from three

superoutbursts, January 1973, February 1980 and December 1982, all observed by Warner at the SAAO^{4,5,13}. Plotting the excess light at mid-eclipse relative to the total light of the system (well away from both eclipses and superhumps), against the superhump phase we obtain Fig. 2a (see Table 1). Note that this has been corrected for the effect of the fading of the non-eclipsed material⁴. The feature remaining corresponds to both the shape and amplitude of the superhump in Z Cha, as is shown in the typical light curve in Fig. 2b. This result demonstrates conclusively that the superhump is uneclipsed in Z Cha at mid-eclipse.

The only other eclipsing SU UMa star, OY Carinae, was observed in superoutburst in January 1980 by Krzeminski. Vogt¹² measured the eclipse depths in magnitudes (relative to the light just out of eclipse) rather than intensities. The result of his method of analysis is that: (1) for eclipsed superhumps the relative eclipse depths will vary in phase with the total superhump light with a maximum at superhump maximum; (2) non-eclipsed superhumps will cause eclipse depths which will vary out of phase with the total superhump light and show a minimum at superhump maximum. The behaviour observed for OY Car¹² is compatible with case (2), and an uneclipsed origin for the superhump light.

Thus, in both observed eclipsing systems the superhump light is visible at mid-eclipse. This is most naturally accounted for as being due to an active, luminous region on the red component, rotating more slowly than the orbital period. The red component then contributes between 20 and 40% of the optical outburst light in an asymmetric 'superspot' region.

If the superhump light originates in the outer rim of the disk it can still be visible at mid-eclipse (except if located close to the line of centres). This light would still be eclipsed, however, and broaden the eclipse, or produce breaks in the eclipse profiles comparable to those visible in quiescent eclipse profiles¹³ (produced in the quiescent case by the orbital hump visible in the light curve of these systems). Because such features are not evident in the superhump eclipse profiles we conclude that the superhump light is entirely uneclipsed.

The observations of Z Cha and OY Car made in superoutburst indicate that the eclipse minima vary from their expected times of minima in a manner dependent on the superhump phase at eclipse^{4,12}. When presented as O-C (the difference between observed, O, and calculated, C, times of mid-eclipse) diagrams of phase of eclipse minimum versus superhump phase,

Fig. 1 Structure of the cataclysmic variable Z Chamaeleontis. Parameters used are those due to Vogt¹², and are the most probable for this system. *a*, An Earth-bound observer's view at mid-eclipse, at superhump phase $\phi_s = 0.35$. The extent of the uneclipsed disk surface is shown shaded above the dashed line. Any disk origin for superhumps must be located in this region at all times. A red-component superhump model has no such limitation. The Roche limit is indicated along with the best observational evidence for the disk tidal radius. *b*, The structure present during outburst. Superhump maximum at $\phi_s = 0$ migrates round in orbital phase through non-synchronism, and possibly additional changes in azimuthal structure. When superhump maximum coincides with mid-eclipse the superhump light remains visible.

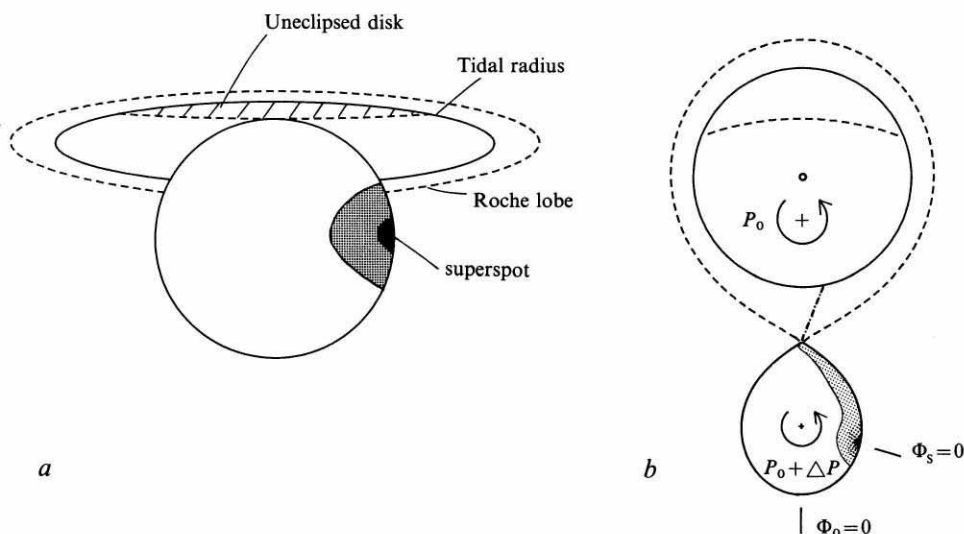


Table 1 Data from Z Cha during three superoutbursts

Superoutburst	Date	Age of superoutburst (days)	Superhump phase	Relative excess light	Refs
January 1973	8	6.0	-0.32	-0.03	5,13
		6.05	-0.35	-0.02	
		6.1	-0.39	-0.02	
		7.1	0.22	0.13	
		7.2	0.15	0.17	
	9	8.1	-0.24	0.06	
		12	9.9	-0.15	0.08
	February 1980	19	2.3	0.48	13
		2.35	0.44	0.01	
		2.4	0.40	0.03	
December 1982	20	3.5	0.02	0.23	4
		3.55	-0.01	0.20	
		6.5	-0.40	0.00	
	23	6.55	-0.44	0.00	
		7.6	0.07	0.16	
	25	8.5	-0.43	0.04	
		14	2.0	-0.48	
	16	2.05	0.48	0.01	
		4.0	-0.43	0.00	
		4.05	-0.46	-0.02	
	17	4.1	-0.49	0.03	
		5.0	0.11	0.22	
	19	5.05	0.07	0.27	
		7.0	0.28	0.15	
		7.05	0.25	0.14	

the times of minima show two effects: an oscillation correlated with superhump phase, and a constant shift. In the data for OY Car, presented in five colours by Vogt¹², both of the above effects are present, and in the case of OY Car find a natural explanation with a red component origin for the superhump light.

A possible explanation of the constant shift has been suggested by Warner⁴. The constant displacement is most naturally due to an asymmetry in the luminosity of the disk due to stream impact and penetration. This displacement of the eclipse minima can be explained by allowing the following lobe of the accretion disk to have a luminosity about 5% greater than the preceding lobe⁴. If spread over a large region of the surface of the disk, this asymmetrical disk light distribution would not be apparent in the light curve except through its shift of mid-eclipse times.

The red-component model necessarily produces an oscillatory effect. The oscillation is due to the geometrical appearance of the red component. The amplitude of the oscillation is determined by the relative shapes and sizes of the eclipses and superhumps which, in turn are determined by the distribution of light over the red component. As the red component rotates within the binary frame, the 'centre of light' of the faintest region oscillates about the line of centres with the superhump period. A synthetic light curve based on appropriate eclipse and superhump parameters generates the theoretical O-C diagram shown in Fig. 3 which mimics very well the observed behaviour for OY Car¹². The variation is caused physically by the changing aspect of the red component and its superhump: if the eclipse minimum ($\phi_0=0$) lies before superhump maximum ($\phi_s=0$) then the asymmetry of the red component advances the eclipse. When the eclipse lies after the superhump then the asymmetry delays the eclipse.

The observed O-C behaviour of Z Cha⁴ does not follow the clean positive/negative oscillation seen for OY Car as the superhump passes through eclipse, but rather a positive shift only. No mechanism is apparent within any proposed model which behaves in this fashion. However, complex structure in both the superspot and disk will distort the behaviour shown in Fig. 3, and low superhump luminosity will reduce its amplitude.

In principle, the structure of the superspot region can be probed using the properties of secondary eclipses. Within the present data no secondary eclipse is apparent. This lack of evidence is not, however, inconsistent with a red-component origin for superhump light. In Z Cha the eclipsed area of the

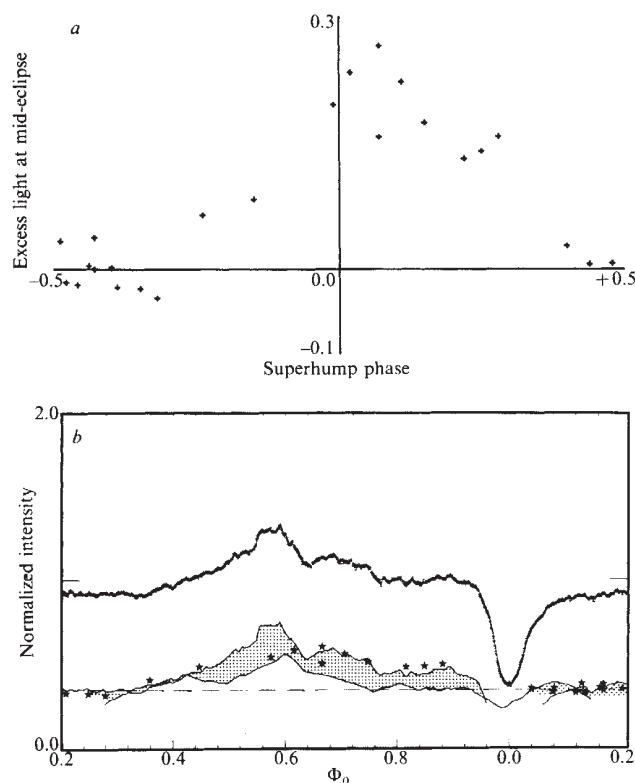


Fig. 2 *a*, The excess light at mid-eclipse in Z Cha as a function of superhump phase. At $\phi_s=0$ this is typically 25% of the background. The additional light is accounted for by the superhumps, as can be seen in *b* where the points in *a* are replotted under a single cycle of the light curve. The dashed line shows the behaviour of eclipsed superhumps, whereas the shaded region indicates the behaviour of uneclipsed superhumps. The shaded area shows the region enveloped by a bright superhump (early outburst) and a faint superhump (late outburst) in which the eclipse minima will lie, in the case of uneclipsed superhumps. Variations in superhump structure lead to some scatter of the points. Clearly the data show that superhumps are not eclipsed at mid-eclipse.

red component is at most 22% (see Fig. 1). If the superspot is concentrated towards the equatorial belt then the depth of the secondary eclipse is as small as 0.06% of the total light for the case of no temperature change in the eclipsed polar region. Even if the superspot is uniformly distributed over one hemisphere the eclipse light is at most 9% of the total light for bright superhumps, and 5% for more typical cases. Similar constraints apply to OY Car. The present superhump light curves show intrinsic variations (due to superspot structure) at levels comparable to the maximum possible secondary eclipse depth. Such eclipses are therefore not readily visible without more data at times of conjunction of superhump maximum with binary phase = 0.5 to reduce the noise.

In both Z Cha and OY Car the red-component mass is estimated¹⁴ as 0.1–0.2 $M_\odot$ (ref. 14). Such main sequence masses imply quiescent temperatures of $\approx 4,000$ K. Initially the superhump luminosity is ≥ 90 times brighter than the quiescent luminosity of the red component. With a red-component origin the superhump must then be emitted from a region at a black-body temperature of at least 12,000 K and probably, with confinement to the equatorial region, superspot temperatures are $\geq 15,000$ K. Such a characteristic temperature for the superspot region is in agreement with the colour temperature deduced from UBV fluxes when the superhump is present in both OY Car¹² and the non-eclipsing system VW Hydri¹⁵.

These temperatures are consistent with predicted responses to dynamical, ionization driven overflow instabilities^{16,17}. As a result of such instabilities, upwelling of material continues until the ionization zones are brought, by adiabatic expansion, to the

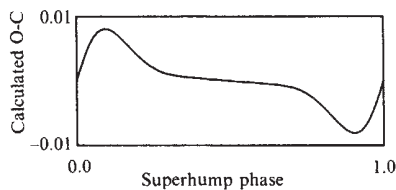


Fig. 3 Model O-C diagram: as the superhump passes through the eclipse, its light alternately shifts the time of minimum to positive, and then negative, phase. The shape and amplitude fit well the observed¹² results for OY Car.

surface. Typical surface temperatures are then 12,000–16,000 K. In normal outbursts these changes will be confined to a region local to the lagrangian point, with typical dimensions of less than a few scale heights ($\leq 0.03 R$ in the region of L_1). However, if non-synchronism spreads the instability along the equatorial belt, a significant fraction of the stellar surface can be affected. The streamlines due to non-synchronous rotation will depend on latitude¹⁸, and lead to further stretching of the superspot region along the longitudinal direction. Azimuthal spatial changes in brightness along the equatorial belt would explain the underlying variation in superhump light. The large opacity changes at the expected superhump temperature $> 15,000$ K may explain the persistent superhump structure on shorter time scales.

The simplest interpretation of the eclipse observations, which has the attraction of being a simple physical structure, is a luminous rotating active region on the equator of the red component. Asynchronous rotation leads to the small period difference between the superhump feature and the binary orbit. Asynchronism may have been driven by the past history of mass and angular momentum exchange (and loss), and gravitational radiation¹⁹. We stress the close similarity between the marching behaviour of superhumps and the migrating wave in the light curve of RS CVn stars²⁰, and the geometrical similarity in their suggested structure²¹.

The origin of the superspot region is consistent with dynamical instability of the red component at L_1 . This powers the superoutburst as fresh unstable material is carried through the L_1 region by slippage of the red component relative to the orbital frame. It remains to be demonstrated that such instabilities will induce disturbances in regions of high latitude. However, pressure gradients will be created over the sub-photospheric equipotential surfaces and may be sufficient to account for the total superhump luminosity by spreading the disturbance to high enough latitudes.

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1. Paradijs, J. van *Astr. Astrophys.* **125**, L16–L18 (1983).
2. Bath, G. T. & Pringle, J. E. *Mon. Not. R. astr. Soc.* **194**, 967–986 (1981).
3. Smak, J. *Publ. astr. Soc. Pacif.* **96**, 5–18 (1984).
4. Warner, B. Preprint (NATO Advanced Studies Institute, Cambridge, 1983).
5. Warner, B. *Mon. Not. R. astr. Soc.* **168**, 235–247 (1974).
6. Vogt, N. *IAU Colloq. No. 42, The Interaction of Variable Stars with their Environment*, 227 (1977).
7. Papaloizou, J. & Pringle, J. E. *Mon. Not. R. astr. Soc.* **189**, 293–297 (1979).
8. Vogt, N. *Astrophys. J.* **252**, 653–667 (1982).
9. Whitehurst, R. *Mon. Not. R. astr. Soc.* **207**, 215–222 (1984).
10. Hensler, G. *Mitt. Astr. Ges.* **60**, 371–374 (1983).
11. Bath, G. T., Edwards, A. C. & Mantle, V. J. *Mon. Not. R. astr. Soc.* **205**, 171–185 (1983).
12. Vogt, N. thesis, Univ. Bochum (1981).
13. Cook, M. C. thesis, Univ. Cambridge (1982).
14. Ritter, H. *Catalogue of Cataclysmic Binaries, Low Mass X-Ray Binaries and Related Objects* (Max-Planck-Institut für Physik und Astrophysik, Munich, 1983).
15. Brunt, C. thesis, Univ. Cambridge (1983).
16. Bath, G. T. *Mon. Not. R. astr. Soc.* **171**, 311–328 (1975).
17. Papaloizou, J. & Bath, G. T. *Mon. Not. R. astr. Soc.* **172**, 339–357 (1975).
18. Campbell, C. G. & Papaloizou, J. *Mon. Not. R. astr. Soc.* **204**, 433–447 (1983).
19. Paczynski, B. *Acta astr.* **31**, 1–12 (1981).
20. Catalano, S., Friona, A. & Rodono, M. *IAU Symp.* No. 88, 405–412 (1979).
21. Hall, D. S. *Publ. astr. Soc. Pacif.* **84**, 323–333 (1972).

No IR burst during a type I X-ray burst from the rapid burster (MXB1730–335)

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The rapid X-ray burster, MXB1730–335, discovered by Lewin *et al.*¹ and identified² with the compact globular cluster Liller 1, produces thousands of X-ray bursts a day when in an active phase. The X-ray bursts have thermal spectra and can be classified into two types by their spectral evolution during burst decay (see ref. 3 and refs therein). Type I bursts normally occur at intervals of hours or days, their spectra soften during decay and their behaviour can be accounted for well by a thermonuclear flash in accreted material on a neutron star. Type II bursts are produced by all other bursters. Type II bursts are unique to the rapid burster, occur at intervals from a few seconds up to 10 min and their spectra do not soften during burst decay. Type II bursts are probably caused by an instability of the accretion flow onto the neutron star. We report here the absence of an IR burst during a type I X-ray burst from MXB1730–335. The upper limit on the IR luminosity during the X-ray burst is 70 times smaller than those of reported IR bursts from this object. This seems to rule out a connection between type I X-ray bursts and the reported IR bursts.

MXB1730–335 became a controversial object when IR bursts were detected. A total of eight IR bursts have apparently been seen by Kulkarni *et al.*⁴ (six bursts with a peak flux of ~ 5 Jy in the H -band) and Jones *et al.*⁵ (two bursts with a peak flux of ~ 7 Jy in the K -band) observing at different times for a total of 8 h. None of these IR variations corresponds with X-ray bursts of the object as the IR bursts they detected were not accompanied by X-ray observations. On the other hand, on nine occasions, an absence of IR bursts during type II bursts was reported by Sato *et al.*⁶ and Glass and Carter (reported by Lawrence *et al.*³). The upper limits placed on IR bursts by these authors are estimated to be more than one order of magnitude smaller than those of the reported IR bursts. Clearly, then, type II bursts are not normally accompanied by IR bursts. Kulkarni *et al.*⁴ suggested that their IR bursts may be associated with type I bursts. During 1980, Lawrence *et al.*³ conducted 180 h of IR observations and did not detect a single IR burst. The upper limit during the main part of these observations was < 0.2 Jy in the K -band, which is more than one order of magnitude smaller than the reported IR bursts. Unfortunately, the rapid burster was in its quiescent state during these IR observations; the Hakucho satellite, which was looking at this source, detected no X-ray bursts³.

On 5 August 1983, the X-ray satellite Tenma detected type I bursts almost every 72 min, showing that the rapid burster was in an active phase. On 20 August, type II burst activity was present, and the burster terminated its activity on 31 August⁷.

We undertook IR observations on 17 August 1983 using K -band ($2.2 \mu\text{m}$) photometry on the 1.9-m telescope of Mount Stromlo Observatory. Sky chopping was used in an east–west direction with a beam throw of 96 arc s at 20 Hz. The diameter of the beam was 30 arc s. The time constant on the lock-in amplifier was set at 0.3 s. No IR burst was detected during the observation time of 90 min between 10 h 54 min and 12 h 23 min (UT) on 17 August 1983. During this period, a typical type I burst was detected by Tenma. This burst began at 12 h 13 min 31 s (UT), peaked 8 s after the onset and faded completely into the noise ~ 80 s after the peak. The peak bolometric X-ray flux was $8 \times 10^{-9} \text{ erg s}^{-1} \text{ cm}^{-2}$, assuming a black-body spectrum (H. Kunieda *et al.*, personal communication). Figure 1 shows the IR raw data recorded on the strip chart at the time corres-

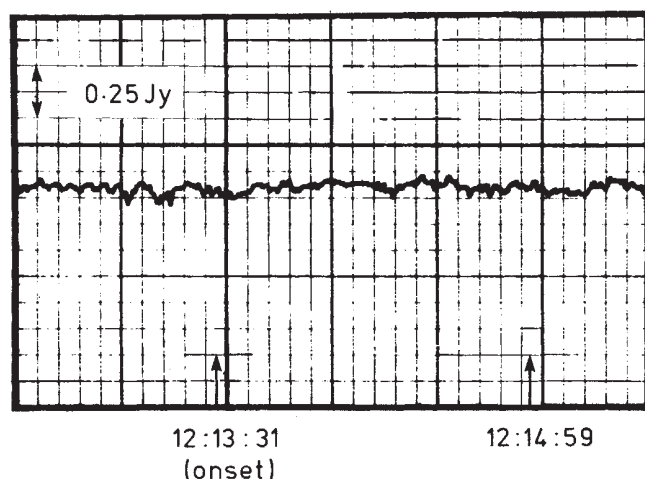


Fig. 1 IR raw data at the K-band corresponding to the time of the X-ray burst, which began at 12 h 13 min 31 s and faded completely into the noise around 12 h 14 min 59 s. The steady level corresponds to the flux from the globular cluster, which has a magnitude of $K = 5.56$ (3.76 Jy) in the 30 arc s aperture.

ponding to the X-ray burst. The steady level corresponds to the flux from the globular cluster. We obtained $K = 5.56$ for the magnitude of the cluster within the 30 arc s aperture, which is consistent with the relation between K -magnitude and aperture size reported by Kleinman *et al.*⁸

The upper limits on variability can be set by using the maximum fluctuations during the observation (times given as UT): $\Delta K < 0.10$ Jy 12 h 13 min 31 s–12 h 14 min 59 s (during the X-ray burst); $\Delta K < 0.25$ Jy 11 h 32 min–12 h 24 min; $\Delta K < 0.4$ Jy 10 h 54 min–11 h 32 min, where 1 Jy corresponds approximately to $K = 7$. Note that the large upper limit of 0.4 Jy in the early part of the observation was probably due to atmospheric conditions and not to luminosity variation of the source.

The upper limit of 0.1 Jy during the type I burst is 70 times smaller than those of reported IR bursts from MXB1730–335. It is unlikely that our time constant of 0.3 s is too long for the detection of IR bursts. Note that Kulkarni *et al.*⁴ detected bursts using a time constant of 0.6 s at the H-band as did Jones *et al.*⁵ with a time constant of 0.3 s at the K-band.

Our negative result seems to rule out the suggestion by Kulkarni *et al.*⁴ that their IR bursts are connected with type I bursts. It is useful to compare our observation with those of other bursters. Coincident X-ray and optical bursts were detected from three type I burst sources, namely 4U/MXB1735–44 (refs 9, 10), MXB1837–05 (ref. 11), and 4U/MXB1636–53 (refs 12, 13). Ten simultaneous X-ray and optical bursts have been observed from 4U/MXB1636–53 by Pedersen *et al.*¹² and Lawrence *et al.*¹³ who found that the optical bursts were delayed by a few seconds relative to the X-ray bursts and that the optical burst flux is too large to explain the optical emission as the tail of the black-body X-ray spectrum. It has been suggested¹² that the matter in the vicinity of the X-ray burster absorbs some fractions of the X rays, is heated to $T \sim 50,000$ K and re-emits the absorbed energy at optical wavelengths.

The 2.2- μ m burst flux which accompanies a type I burst from the rapid burster can be estimated on the assumption that type I bursts from the rapid burster are caused by the same physical process as that suggested for 4U/MXB1636–53; the ratio of the optical peak flux at 3,350–5,250 Å to the bolometric X-ray peak flux is 10^{-3} , after correcting for an interstellar extinction of $A_v = 2.5$ (ref. 11) and assuming that the material heated by the X rays emits a black-body spectrum with $T = 50,000$ K at optical and IR wavelengths¹³. The bolometric X-ray burst peak flux of 8×10^{-9} erg s⁻¹ cm⁻² measured by Tenma predicts an apparent 2.2- μ m peak flux of 3×10^{-14} erg s⁻¹ μ m⁻¹ cm⁻² corresponding to 5×10^{-5} Jy assuming an interstellar extinction of

$A_K = 1.1$ (ref. 8). This is three orders of magnitude smaller than our upper limit of 0.1 Jy during the type I burst. Hence, if a fraction of type I bursts are accompanied by the reported IR bursts, the mechanism should be quite different from that suggested to explain the well known optical bursts of normal bursters. We note that Lewin *et al.*¹⁴ have also reported the absence of an IR burst during a type I X-ray burst from Ser X-1.

Jones *et al.*⁵ pointed out that neither type I nor type II bursts fit the reported IR bursts, whose pattern of occurrence differs from those of X-ray bursts. We conclude that type I bursts do not seem to be accompanied by the reported IR bursts. However, we cannot rule out the possibility that a fraction of type I bursts may be accompanied by the reported IR bursts, as we have only observed one type I burst which shows no IR counterpart.

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1. Lewin, W. H. G. *et al. Astrophys. J. Lett.* **207**, L95–L99 (1976).
2. Liller, W. *Astrophys. J. Lett.* **213**, L21–L23 (1977).
3. Lawrence, A. *et al. Astrophys. J.* **267**, 301–309 (1983).
4. Kulkarni, P. V., Ashok, N. M., Apparao, K. M. V. & Chitre S. M. *Nature* **280**, 819–820 (1979).
5. Jones, A. W. *et al. Nature* **283**, 550–551 (1980).
6. Sato, S., Kawara, K., Kobayashi, Y., Maihara, T. & Okuda, H. *Nature* **286**, 688–689 (1980).
7. Kunieda, H. *et al. Preprint*, Nagoya Univ. (1984).
8. Kleinmann, D. E., Kleinmann, S. G. & Wright, E. L. *Astrophys. J. Lett.* **210**, L83–L86 (1976).
9. Grindlay, J. E. *et al. Nature* **274**, 567–568 (1978).
10. McClintock, J. E. *et al. Nature* **279**, 47–49 (1979).
11. Hackwell, J. A. *et al. Astrophys. J. Lett.* **223**, L115–L119 (1979).
12. Pedersen, H. *et al. Astrophys. J.* **263**, 325–339 (1982).
13. Lawrence, A. *et al. Astrophys. J.* **271**, 793–803 (1983).
14. Lewin, W. H. G. *et al. Nature* **287**, 27–28 (1980).

First optical detection of atomic deuterium in the upper atmosphere from Spacelab 1

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Measurements of the deuterium-to-hydrogen ratios (D/H) in the fields of meteorology^{1,2}, tropospheric^{3,4} and stratospheric chemistry^{5,6}, planetology^{7,8} and cosmogony^{8,9} have previously been performed on molecules in which one hydrogen atom is replaced by one deuterium atom. We report here the first detection of isolated deuterium atoms in the Earth's upper atmosphere through their resonantly scattered Lyman- α emission. An atmospheric deuterium emission of 330 R (1 R = 10^6 photons cm⁻² s⁻¹) is observed for a tangent altitude of 110 km during Spacelab 1 mission launched on 28 November 1983. The (D/H) ratio of 3×10^{-4} is slightly enriched over the seawater value of 1.6×10^{-4} .

In the terrestrial upper atmosphere, hydrogen atoms are illuminated by the intense solar Ly α line at the resonance wavelength of 121.566 nm in the far UV. The solar Ly α is so wide that it can also illuminate deuterium atoms at the resonance wavelength of 121.533 nm. Through resonant scattering, hydrogen and deuterium can re-emit their Ly α line. Although hydrogen Ly α emission has been observed many times from space, deuterium Ly α has not been detected for two reasons. First, the (D/H) ratio in seawater is only 1.6×10^{-4} . Second, the spectral distance between the two Ly α lines is quite small.

However, the resonance lines of both isotopes are well separated, as their spectral distance of 0.033 nm is much larger than their thermal width of ~ 0.002 nm. Consequently, the spectral

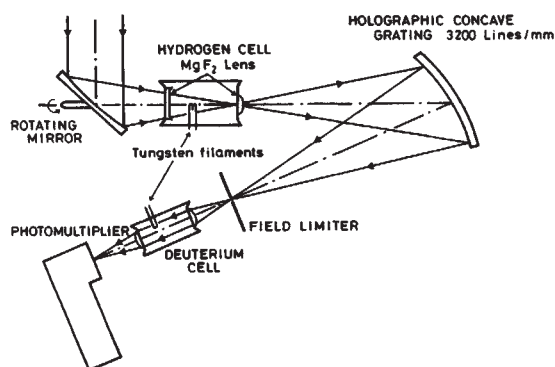


Fig. 1 Optical diagram of experiment 1ES017. The mass of the instrument is 12.5 kg. The dimensions are 685 × 300 × 320 mm and the average power required is 30 W.

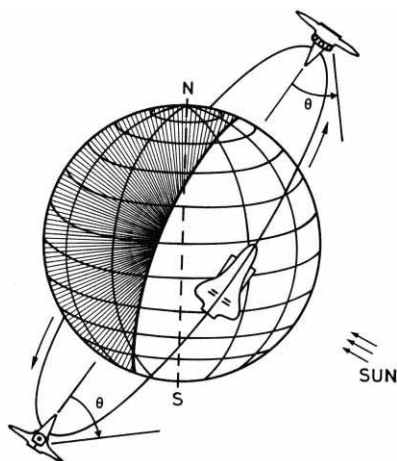


Fig. 2 Geometrical configuration for bright limb observations from an altitude of 250 km. The orbit altitude sketch is greatly exaggerated in respect to the Earth's radius.

resolution needed to distinguish them would have meant using a prohibitively large and heavy classical spectrometer on this first Spacelab mission. We have, therefore, designed and built a lightweight spectrophotometer (Fig. 1) equipped with molecular hydrogen and deuterium absorption cells to suppress selectively one or two of the natural emissions of both isotopes H and D recorded by the instrument. Atmospheric light is collected with a small parabolic off-axis mirror, rotating around one axis to provide a vertical scan of the emission around the limb. The light goes through the hydrogen cell, which is transparent to Ly α when it is not activated, and enters a spectrometer with a single concave holographic grating. A hole at the exit defines both the 2.5° circular field of view of the instrument and a spectral bandwidth of ~ 4.5 nm centred at Ly α . Thus, the strong atmospheric emission line of atomic oxygen at 130.4 nm is eliminated. The light is then focused on the photomultiplier window through the deuterium cell equipped with MgF₂ lenses. Photons are counted in two counters, sequentially opened in phase with a 5-Hz modulation of the deuterium cell: one counter C₁ is devoted to photons when the deuterium cell is off, the other counter C₂ is devoted to photons when the deuterium cell is activated, that is, when molecular deuterium is dissociated into atoms in the cell.

Fortunately, the atomic hydrogen Ly α emission is saturated, when looking towards the limb, because of the large H column density in the line-of-sight, whereas the optically thin emission I_D of D atoms is proportional to the D column density. As a result, the intensity ratio I_D/I_H is much larger than the (D/H) ratio.

The geometry of observations is illustrated in Fig. 2. The limb is observed from an altitude of 250 km at right angles to the

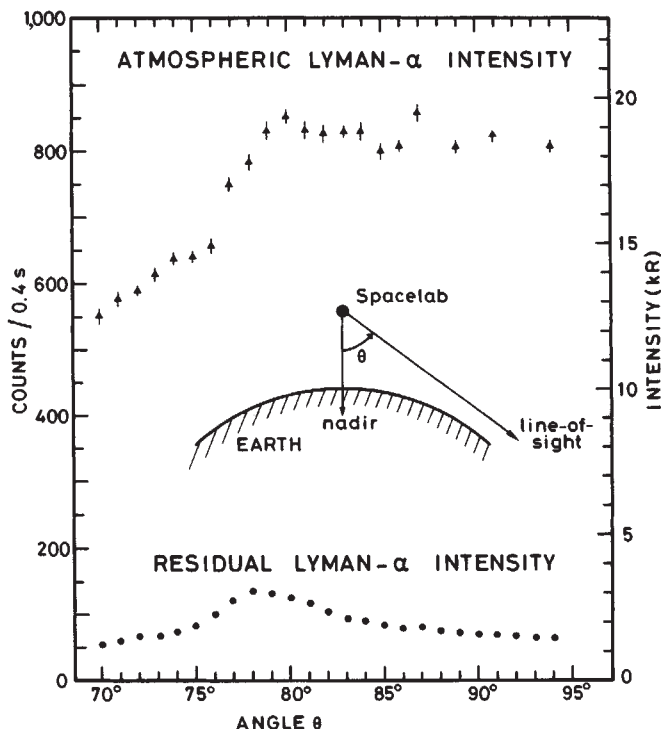


Fig. 3 Measured atmospheric Ly α intensity is shown in the upper part. The residual Ly α intensity indicated in the lower part corresponds to the intensity which is not absorbed when the hydrogen cell is turned on. The left-hand ordinate corresponds to the photomultiplier count rate which is converted into kR in the right-hand scale. The geometrical significance of the observational angle θ is shown in the centre.

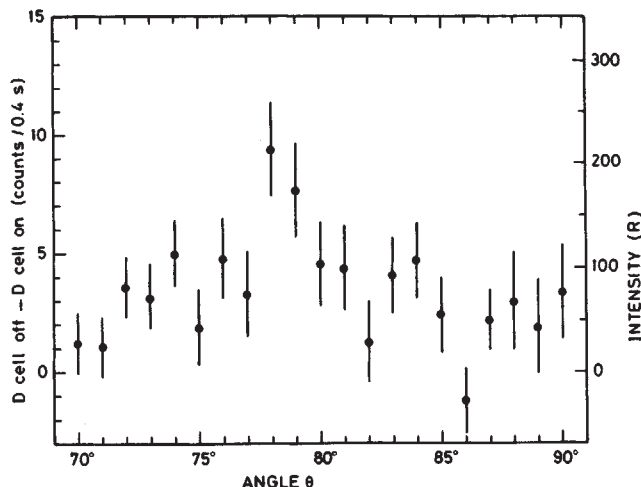


Fig. 4 Atmospheric deuterium Ly α intensity absorbed by the activated deuterium cell. During these measurements the hydrogen cell is permanently on whereas the deuterium cell is alternatively switched on and off. The measured points represent an average of 74 angle scans. Angle θ has the same significance as in Fig. 3.

orbital plane, in order to cancel the Doppler shift associated with the 8 km s⁻¹ space shuttle velocity. Otherwise, the absorption cells would have had little or no effect on the atmospheric H and D emissions.

The measured intensity, which essentially results from H Ly α emission, is displayed in the upper part of Fig. 3 as a function of the angle θ with the local vertical. When approaching the limb from below, the H Ly α increases up to the point where the line-of-sight passes above 110 km altitude, corresponding to $\theta = 78^\circ$. Above this height, absorption of Ly α emission by atmospheric molecular oxygen becomes negligible and the Ly α intensity reaches 20 kR.

When a tungsten ribbon is heated electrically in the H_2 cell, an optical thickness $\tau_H \approx 600$ of atomic H is created inside the cell, which absorbs a large fraction (80–90%) of the total signal. The H_2 cell acts as a 'negative' filter and lets through every photon with a wavelength lying outside the absorption profile, which has an equivalent width of 0.006 nm. The transmitted signal is recorded by counter C_1 and shown at the bottom of Fig. 3. It contains mainly H Ly α photons from the linewings, which extend slightly outside the negative filter because of saturation effects.

A small contribution of atmospheric deuterium Ly α emission can then be identified by using the deuterium cell, which creates an optical thickness $\tau_D \approx 1.5$, representing a negative filter selective for D Ly α emission. For $\tau_D = 1.5$, it can be shown that $\sim 60\%$ of the deuterium intensity I_D is absorbed in the cell, and the difference between the two counters $C_1 - C_2$ represents a measurement of $0.6I_D$. This absorbed intensity is plotted both in counts per 0.4 s and in Rayleighs in Fig. 4.

Seventy-four scans around the limb were averaged and binned in steps of $\Delta\theta = 1^\circ$ to obtain the curve in Fig. 4, representing ~ 100 min of measurements spread over three orbits and a large range of latitudes. The size of the error bar attached to $C_1 - C_2$ is 2.5 times the error bar attached to counts C_1 , which is $(C_1/n)^{1/2}$. Without measurable deuterium emission I_D , the points would be equally positive or negative, which is not the case. Except for one value at $\theta = 86^\circ$, all points are positive, which means that atmospheric deuterium Ly α emission has been unambiguously detected.

A narrow maximum of deuterium emission is found around $\theta = 78^\circ$, at an altitude of ~ 110 km. This limb brightening effect is expected from the optically thin deuterium emission, when the line-of-sight passes just above the Ly α limb created by atmospheric O_2 absorption. As the field of view of the instrument is 2.5° , it means that the observable deuterium layer is even smaller than what is shown in Fig. 4. However, we can produce a preliminary estimate of the deuterium quantity corresponding to our observations. Because the emitted deuterium Ly α intensity I_D is 330 R, the column number density N_D and the intensity I_D are linked by:

$$I_D(R) = 10^{-6} \sigma F_s N_D = 10^{-6} g N_D$$

where F_s is the solar flux¹⁰ of 2.8×10^{12} photons $cm^{-2} s^{-1} nm^{-1}$. Because the integrated cross-section of D atoms is $\sigma = 5.43 \times 10^{-16} cm^2 nm$, the excitation rate of D atoms by solar photons is $g = \sigma F_s = 1.52 \times 10^{-3} s^{-1}$, and these photons are scattered isotropically.

For $I_D = 330$ R, $N_D = 2.2 \times 10^{11}$ atoms cm^{-2} along the line-of-sight tangent at 110-km altitude. We have compared this integrated density to a vertical distribution model depending mainly on one parameter, the eddy diffusion coefficient K , which indicates roughly the altitude of the homopause level, where the atmosphere goes from a well mixed regime to one in which all gases are distributed according to their own scale height and mass. It has been shown¹¹ that the (D/H) ratio is sensitive to this value of K and also to the exospheric temperature T . Our value of $N_D = 2.2 \times 10^{11}$ atoms cm^{-2} corresponds to a model with a thermopause temperature of 900 K in which the number density is $2.5 \times 10^3 cm^{-3}$ at 110 km of altitude, and the (D/H) ratio is of the order of 3×10^{-4} . This modest enrichment by a factor of 2 corresponds to a height-independent eddy diffusion coefficient of $1.3 \times 10^6 cm^2 s^{-1}$.

Our deuterium observations were hampered during this Spacelab mission by two problems. First, the sensitivity of our first flight model was a factor of 4 below its nominal value, while a second unit unfortunately achieved nominal sensitivity too late to be accommodated in the Spacelab payload. Second, the orbit, because of date and time of launch, soon shifted towards a position near the terminator, where excitation of deuterium atoms by solar Ly α photons is decreased by a larger O_2 column density. As a result, we could observe deuterium emission only during the first 24 h, although the mission lasted for 10 days. A second mission could help to establish a variation

of the homopause level with latitude, a major atmospheric objective.

Nevertheless, we have demonstrated that the natural Ly α emission of deuterium atoms can be used to evaluate their number density in the Earth's atmosphere by a remote-sensing technique.

Finally, this remote-sensing technique could be used with the Space Telescope or space probes for the other planets, in particular for Venus and Mars, where the (D/H) ratio is unknown. For Venus, a (D/H) ratio of 1.6×10^{-2} was found⁷ in a cloud droplet sucked by a neutral mass spectrometer on board the large probe of Pioneer Venus mission. Such a high value can be explained only by a fantastic differential escape of both isotopes^{12,13}, the heavier D atoms being left behind in the atmosphere of Venus. The quantity of water at the origin of Venus was at least 100 times larger⁷ than it is now.

Such an important clue about the history of water on Venus deserves further investigation. We have demonstrated the feasibility of the deuterium Ly α detection technique. It can now be applied to detailed terrestrial and planetary studies.

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1. Lawrence, J. R., Gedzelman, S. D., White, J. W. C., Smiley, D. & Lazov, P. *Nature* **296**, 638–640 (1982).
2. Leguy, C., Rindsberger, M., Zangwil, A., Issar, A. & Gat, J. R. *Isot. Geosci.* **1**, 205–218 (1983).
3. Ehalt, D., Roether, W. & Stich, W. Z. *Naturforsch.* **21a**, 1703–1709 (1966).
4. Ehalt, D. & Ostlund, H. G. *J. geophys. Res.* **75**, 2323–2327 (1970).
5. Scholz, T. G., Ehalt, D. H., Heidt, L. E. & Martell, E. A. *J. geophys. Res.* **75**, 3049–3054 (1970).
6. Pollock, W., Heidt, L. E., Lueb, R. & Ehalt, D. H. *J. geophys. Res.* **85**, 5555–5568 (1980).
7. Donahue, T. M., Hoffman, J. H., Hodges, R. R. Jr & Watson, A. H. *Science* **216**, 630–633 (1982).
8. Gautier, D. & Owen, T. *Nature* **304**, 691–694 (1983).
9. Geiss, J. & Reeves, H. *Astr. Astrophys.* **93**, 189–199 (1981).
10. Lemaire, P. et al. *Astrophys. J. Lett.* **223**, L55–L58 (1978).
11. Kockarts, G. *Space Res.* **12**, 1047–1050 (1972).
12. Kasting, J. F. & Pollack, J. B. *Icarus* **53**, 479–508 (1983).
13. Kumar, S., Hunten, D. M. & Pollack, J. B. *Icarus* **55**, 369–383 (1983).

Crustal evolution in north-east and east Africa from model Nd ages

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The relationships between the various crustal provinces that comprise north-east and east Africa are far from clear and reflect diverse tectonic and magmatic processes which took place during the Proterozoic. Here we present the results of an Nd isotope study on the major rock units of the Pan-African (1,100–500 Myr BP) terrane. Charnokites from Jabel Uweinat, a basement inlier at the junction of Egypt, Libya and the Sudan, yield middle Archaean model Nd ages, whilst model ages of $<1,200$ Myr have been obtained in a belt from the Eastern Desert of Egypt to north-west Kenya. This, therefore, represents a westward extension of the juvenile Pan-African crust of the Arabian Shield (Fig. 1). The shield itself is characterized by comparatively rapid crustal growth¹, probably in an accreting island arc environment². Between the two areas, there is a marginal zone of Pan-African rocks with relatively low $\epsilon_{Nd}(T)$ values (see Table 1) due to increased contributions from the pre-Pan African crust. Overall, the Pan-African rocks from north-east and east Africa and those from the Damara of Namibia³ exhibit a wide range of $\epsilon_{Nd}(T)$ from +7.5 to –18.0 which reflects regional changes in tectonic style and is not readily reconciled with simple models for the evolution of average continental crust.

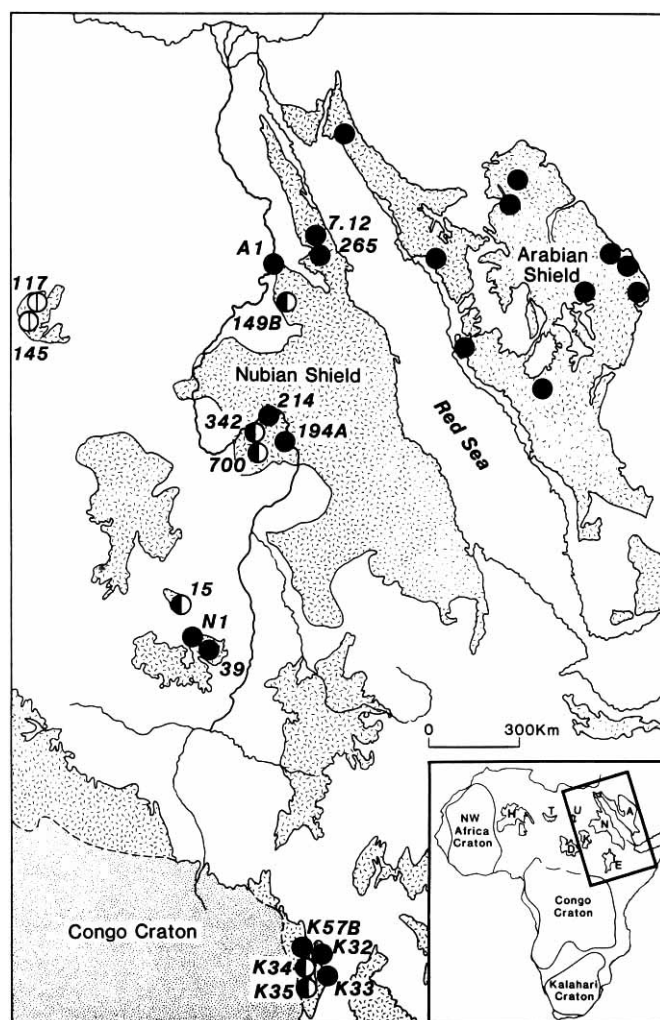


Fig. 1 Sample location map showing extent of basement outcrop and distribution of Nd model ages in north-east and east Africa and Arabia. ●, 800–1,300 Myr; ○, 1,600–1,900 Myr; □, 3,000–3,200 Myr. Inset shows the location of the study area relative to African cratons of Archaean age, and basement inliers at Hoggar (H), Tibesti (T), Jabel Uweinat (U), Darfur (D), Kordofan (K) and western Ethiopia (E); also shown are Nubian (N) and Arabian (A) shields.

The Karkur Murr charnockites from Jabel Uweinat (Fig. 1) form the oldest known basement in north-east Africa^{4,5} and two samples have model Nd ages, relative to depleted mantle⁶, of 3,000 and 3,200 Myr (Fig. 2, Table 1). This confirms their Archaean age, while their low $\epsilon_{\text{Nd}}(T)$ and high $\epsilon_{\text{Sr}}(T)$ values indicate that the event dated at $2,617 \pm 21$ Myr by Rb/Sr whole rock analyses⁵ involved considerable crustal reworking. Figure 2 also illustrates that none of the other samples from this study has a uniquely Archaean source. Most samples from Egypt and the Sudan have Nd model ages between 750 and 1,150 Myr. They are therefore isotopically indistinguishable from the juvenile Pan-African crust of the Arabian Shield^{1,7}. The amphibolite from the Marich area (K57B) of north-west Kenya^{8,9} and the foliated granodiorite from the Abu Harik complex (194A) of the Bayuda Desert of the western Nubian Shield¹⁰ lie just above the evolution path for average depleted mantle, as do some of the Pan-African rocks from Arabia^{1,7}. The metasediment (265) and the foliated granite (7.12) from the Hafafit region of Egypt represent the highest grade of metamorphism in the Eastern Desert¹¹ and lie well within this age group, indicating that, despite their strong deformation, they are Pan-African in age and were derived from juvenile crust. The Aswan granite (A1) has high initial $\epsilon_{\text{Nd}}(T)$ (+2.3) and low initial $\epsilon_{\text{Sr}}(T)$ (−8.5), values similar to those reported from other

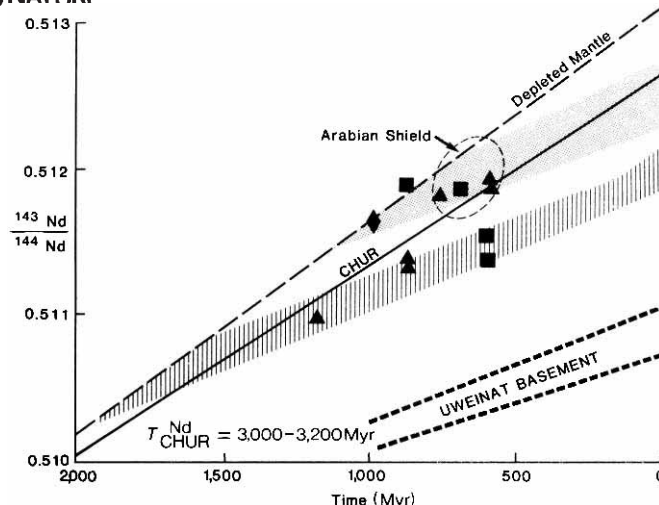


Fig. 2 Variations in initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios in samples from north-east and east Africa. The stippled region encloses the evolution lines for samples 265, 7.12, A1, 194A, 214, N1, 39, K32, K33 and K57B. The hatched region encloses the evolution lines for samples 342, 700, 15, 149B, K34 and K35. Evolution paths truncated by inferred light REE-depleted mantle⁶. ■, Granitic and meta-igneous samples; ▲, metasediments; ◆, amphibolites. Field of Arabian data taken from ref. 1.

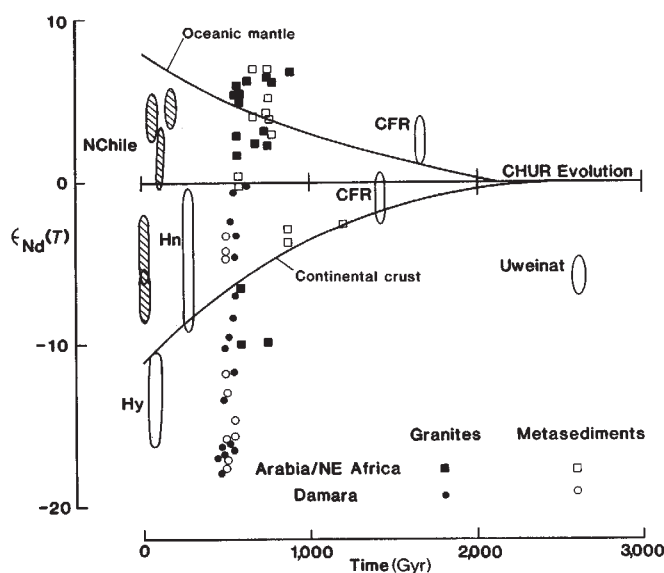


Fig. 3 Mixing model curves for evolution of oceanic mantle and continental crust assuming that the last two orogenies contributed to continental crust²⁷. Pan-African data are taken from north-east Africa, Arabia¹ and Damara^{3,30}. Also shown are data from northern Chile^{29,31}, Himalayas (Hy)²⁷, the Hercynian of south-west Europe (Hn)^{27,28} and the Columbia Front Range (CFR)⁶.

younger granites in the Eastern Desert¹² and to many recent mantle-derived rocks. However, this lack of evidence for at least a significant contribution from older crustal material is apparently at variance with published radiogenic lead isotope results^{13,14}. This may simply reflect differences between the samples analysed; other Rb–Sr studies¹⁵ have reported initial $\epsilon_{\text{Sr}}(T)$ as high as +70, implying that the Aswan granite was isotopically heterogeneous at the time of emplacement. Alternatively, radiogenic lead could have been introduced by subduction processes, as has been recently demonstrated in the Lesser Antilles¹⁶ where samples with $\epsilon_{\text{Nd}}(T) = +2$ have $^{207}\text{Pb}/^{204}\text{Pb} =$

Table 1 Sr and Nd data from localities in north-east Africa

Locality	Lithology	Sample	Age (Myr)	Ref.	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{87}\text{Rb}/^{86}\text{Sr}$	$\epsilon_{\text{Sr}}(T)$	$^{143}\text{Nd}/^{144}\text{Nd}$	Sm	Nd	$^{147}\text{Sm}/^{144}\text{Nd}$	$\epsilon_{\text{Nd}}(T)$	T_{DM} (Myr)
Jabel Uweinat	Charnockite (Karkur Murr)	117	2,617±21	5	0.8037	2.515	+99.6	0.51073±3	2.33	14.2	0.099	-4.4	3,000
	Charnockite (Karkur Murr)	145	2,617±21	5	0.8928	4.915	+74.4	0.51103±2	4.63	23.5	0.119	-5.3	3,200
Eastern Desert	Metasedimentary pebble (Hafafit)	265	—	—	—	—	—	0.51259±2	16.47	84.7	0.118	+6.6*	750
	Metapelite (Wadi Haimur)	149B	1,195	19	—	—	—	0.51200±3	6.80	32.2	0.128	-1.9	1,800
	Foliated granite (Gabal Zabara)	7.12	—	—	—	—	—	0.51255±1	3.43	17.0	0.122	+5.4*	850
	Granite (Aswan)	A1	680±10	13	0.7033†	—	-8.5	0.51232±1	11.20	68.9	0.098	+2.3	950
Bayuda Desert	Quartzofeldspathic metasediment (Rahaba)	342	874±33	10	—	—	—	0.51206±1	2.56	13.0	0.119	-2.6	1,500
	Quartzofeldspathic metasediment (Rahaba)	700	874±33	10	—	—	—	0.51202±1	2.74	14.0	0.118	-3.3	1,550
	Foliated granodiorite (Abu Harik)	194A	898±51	†	0.7035	0.067	-13.8	0.51277±2	2.18	8.6	0.153	+7.5	750
	Quartzite (Abu Hamed)	214	761±22	†	0.7394	3.356	-12.0	0.51240±2	0.27	1.4	0.117	+3.1	1,050
	Foliated biotite granite (Rashad)	N1	—	—	—	—	—	0.51229±3	4.55	26.9	0.102	+2.2*	1,000
Central Sudan	Foliated granodiorite (Abbasiya)	39	—	—	—	—	—	0.51237±2	10.03	57.4	0.106	+3.5*	950
	Muscovite granite (El Obeid)	15	—	—	—	—	—	0.51214±3	1.99	5.95	0.202	-10.3*	—
	Granite (Marich)	K34	593±49	†	0.7189	1.382	+45.9	0.51202±2	0.48	2.38	0.122	-6.4	1,650
North-west Kenya	Granite (Marich)	K35	593±49	†	0.7127	0.692	+40.7	0.51185±1	0.21	1.03	0.123	-9.9	1,950
	Metapelite (Sekerr)	K32	584±25	†	0.7068	0.310	+3.2	0.51239±2	1.33	6.33	0.127	+0.3	1,100
	Metapelite (Sekerr)	K33	584±25	†	0.7146	1.259	+1.7	0.51237±3	1.09	5.17	0.127	-0.1	1,150
	Amphibolite (Sekerr)	K57B	982±40	†	0.7112	0.589	-8.3	0.51243±3	5.60	28.20	0.120	+5.6	1,000

$\epsilon_{\text{Nd}}(T)$ is the deviation from the value expected in a chondritic reservoir (CHUR) at time T , and is defined as $\epsilon_{\text{Nd}}(T) = 10^4 \left[\frac{^{143}\text{Nd}/^{144}\text{Nd}_{\text{rock}}(T)}{^{143}\text{Nd}/^{144}\text{Nd}_{\text{CHUR}}(T)} - 1 \right]$ where $^{143}\text{Nd}/^{144}\text{Nd}_{\text{CHUR}}(T) = 0.51264 - 0.1967[(\exp \lambda_{\text{Sm}} T) - 1]$. T_{DM} calculated using values from ref. 6.

* Assuming age of 750 Myr. † A.C.R., unpublished data. ‡ Isochron initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio.

15.73. Farther south, the quartzite from the Abu Hamed series of the Bayuda Desert¹⁰ (214), and the Rashad granite (N1) and the Abbasiya granodiorite (39) from the Nuba Mountains of southern Kordofan, central Sudan¹⁷ are derived from the source regions of Pan-African age.

The third group of results provides Nd model ages between 1,500 and 2,000 Myr. Model ages in this range were not observed in a recent Nd isotopic study of the Arabian Shield¹ (Fig. 1). In general, such isotopic characteristics may be derived either from a source region of this age or from mixed sources including, presumably, both Archaean and Pan-African material. In the case of the Marich granite (K34, K35), which is exposed 20 km east of the margin of the Congo Craton in north-west Kenya^{8,9}, its high $\epsilon_{\text{Sr}}(T)$ and low $\epsilon_{\text{Nd}}(T)$ values confirm that it was derived from pre-existing continental crust, and its model age implies that the source included a pre-Pan-African component. The variation in model age between the two samples indicates that isotopic homogeneity was not reached during anatexis, consistent with the scatter of the Rb/Sr whole rock analyses (mean sum of weighted deviates = 51; A.C.R., unpublished data). The Sekerr metasediments (K32, K33) into which the Marich granite is intruded have model ages of 1,150–1,100 Myr, implying that little, if any, of the sediment was derived from the Archaean craton to the west. The muscovite granite (15) from the El Obeid area of Kordofan Province, Central Sudan, also has a low present day $^{143}\text{Nd}/^{144}\text{Nd}$ ratio relative to that of granite of known Pan-African age, but its model age cannot be calculated since its evolution path closely parallels CHUR. Its flat rare earth element (REE) profile is typical of peraluminous granites and indicates an increase in Sm/Nd during melting. In the simplest model, if it is assumed that melting occurred during the Pan-African from a single source with a Sm/Nd ratio similar to that of average crust, the model Nd age of the source would be at least 2,000 Myr. Thus both the El Obeid and the Marich peraluminous granites contain significant contributions from the pre-Pan-African crust.

Similarly, some of the source regions for the Rahaba metasediments in the Bayuda Desert¹⁰ (342, 700), initially mapped as grey gneisses and identified as the oldest Pan-African units in their area¹⁸, and for the amphibolite facies metapelites in southern Egypt¹⁹ (149B) must also predate the major Pan-African crust-forming episodes. These data support the existence of early Proterozoic crust, but argue against the presence of Archaean basement, in the western Nubian Shield and in the Kordofan inlier^{20,21}.

Nd-isotope studies have therefore identified important differences in the character of the Pan-African terrane across Arabia and north-east Africa. The samples analysed from Arabia all have positive $\epsilon_{\text{Nd}}(T)$ values and none of them is likely to have crustal precursors older than 1,200 Myr (ref. 1) (Figs 1, 2). Similar results were obtained on samples along a belt from southern Egypt to north-west Kenya, but to the north-west there is a shift to lower ϵ_{Nd} and hence older model Nd ages, culminating in the Archaean ages from Jabel Uweinat. Thus an area of predominantly juvenile Pan-African crust is bounded by one in which rocks apparently formed during the Pan-African event nonetheless have model Nd ages of 1,500–2,000 Myr. However, these rocks are (1) metasediments, and (2) peraluminous granites chosen from areas of higher metamorphic grade as probable crustal melts. There is no evidence of a decrease in $\epsilon_{\text{Nd}}(T)$ in subduction-related magmas (such as A1 and 194A) near the margins of the craton, suggesting that the $\epsilon_{\text{Nd}}(T)$ values of these rocks have not been affected by contamination with upper crustal material. Furthermore, this study has failed to identify any Archaean crust to the east of Jabel Uweinat, and since the Egyptian and Sudanese samples are tightly grouped into two discrete suites, the data suggest that the Archaean basement exposed at Jabel Uweinat was rimmed by younger pre-Pan-African crust of 1,500–2,000 Myr. Two upper intercept U–Pb zircon ages from distinct metasediments in the Eastern Desert—a granite pebble from a conglomerate in Wadi Mobarak ($1,960 \pm 200$ Myr)²² and an arkosic metasediment from Wadi

Nugrus ($1,700 \pm 40$ Myr)²³—support such a view. Moreover, in the Hoggar region of north-west Africa a widespread 'Eburnean' event of granulite metamorphism at $2,000 \pm 100$ Myr has been identified²⁴; in the Jabel Uweinat inlier crustal anatexis has been recorded at $1,800 \pm 40$ Myr (ref. 5); zircons from Phanerozoic sandstones in Tunisia indicate an age of $1,750 \pm 100$ Myr (ref. 25) and in the Iringa region on the edge of the Mozambique Belt in Tanzania isotopic ages of $1,850 \pm 100$ Myr are common²⁶. Thus it is not unreasonable that crust of this age was sampled by sediments in the Eastern Desert and the Bayuda Desert, and by anatexic melts in central Sudan and north-west Kenya.

The Pan-African event represents a critical stage in the evolution of both large-scale tectonic processes and the continental crust. The volume of stable continental crust has increased, probably at varying rates, since $\sim 4,000$ Myr. Since the stabilization of such low Sm/Nd, high Rb/Sr crust is held to be primarily responsible for the high Sm/Nd, low Rb/Sr characteristics of most of the upper mantle, the Nd- and Sr-isotope ratios of the average crust and average upper mantle have also diverged with time²⁷ (see Fig. 3). Successively younger orogenic episodes, and particularly those over the last 1,000 Myr, should therefore have had more opportunity to remobilize crust which was old enough to have developed distinctive isotope ratios.

Figure 3 summarizes data on granitic rocks from Columbia Front Range⁶, the Hercynian of Europe^{27,28}, the Himalayas²⁷, and on both extrusives and intrusives from a section across the northern Chilean Andes²⁹. Igneous rocks older than 2,000 Myr tend to have initial Nd- and Sr-isotope ratios close to those of the bulk Earth²⁷ (although Jabel Uweinat offers an interesting exception) while the Phanerozoic examples have variable and often lower $\epsilon_{Nd}(T)$ values. Thus the young granitoids contain a greater contribution from significantly older crust, either because little old crust survived early in Earth's history and/or younger granites were generated by different processes. Also shown in Fig. 3 are analyses of Pan-African granitoids and metasediments from Arabia¹, north-east Africa and the Damara Belt in Namibia^{3,30} with calculated curves for the evolution of average continental crust and upper mantle²⁷. Superficially the wide range of $\epsilon_{Nd}(T)$ in the Pan-African is difficult to reconcile with the curve for the evolution of average crust, but that in part depends on the processes responsible for $\epsilon_{Nd}(T)$ values in orogenic rocks.

The range of $\epsilon_{Nd}(T)$ in the Pan-African samples reflects differences in the tectonic regime, and in the age of the pre-existing crust. Areas of significant crustal accretion have $\epsilon_{Nd} = +2$ to $+7$, and it requires a marked change in thermal regime, with an increase in metamorphic grade usually associated with crustal thickening, to generate predominantly crustal-derived magmas with low $\epsilon_{Nd}(T)$ (for example, the Damara³⁰). In this case $\epsilon_{Nd}(T)$ values primarily reflect the age of the pre-existing crust. But crustal thickening is typically a late and comparatively short-lived stage in an orogenic cycle, and thus the low $\epsilon_{Nd}(T)$ granites are often trivial volumetrically. For example, a detailed section across the northern Chilean Andes²⁹ has shown that from the start of the present orogenic cycle at ~ 185 Myr until ~ 20 Myr the intrusive and extrusive rocks had $\epsilon_{Nd}(T) = +6$ to 0, and it is only over the past 10–15 Myr, since the development of thick crust, that low $\epsilon_{Nd}(T)$ rocks have been generated³¹ (see also Fig. 3). Similarly, in the Himalayas subduction zone related magmatism associated with the closure of Tethys may have occurred since the Upper Triassic, but the low $\epsilon_{Nd}(T)$ rocks on Fig. 3 are small-volume, post-collisional crustal melts again formed in response to crustal thickening.

Thus, while crust-forming processes tend to generate magmas with $\epsilon_{Nd}(T) = +7$ to $+2$ (as in northern Chile and Arabia, Fig. 3), the Nd-isotope composition of low $\epsilon_{Nd}(T)$ granites is, like that of sediments, primarily an indication of the age of the local pre-existing crust. As demonstrated here for the Pan-African, the $\epsilon_{Nd}(T)$ values of magmatic rocks can vary dramatically even in broadly contemporaneous orogenic areas, depending on both the age of the basement and the tectonic environment.

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1. Duyverman, H. J., Harris, N. B. W. & Hawkesworth, C. J. *Earth planet. Sci. Lett.* **59**, 315–326 (1982).
2. Gass, I. G. *J. geol. Soc. Lond.* **134**, 129–138 (1977).
3. Hawkesworth, C. J., Kramers, J. D. & Miller, R. McG. *Nature* **289**, 278–282 (1981).
4. Klerkx, J. in *The Geology of Libya* Vol. 2 (ed. Salem, M. J.) 901–906 (Academic, New York, 1980).
5. Klerkx, J. & Deutsch, S. *Mus. R. Afr. centr. Tervuren Dept. Géol. Min., Rapp. ann.* **1976**, 83–94 (1977).
6. De Paolo, D. J. *Nature* **291**, 193–196 (1981).
7. Bokhari, F. T. & Kramers, J. D. *Earth planet. Sci. Lett.* **54**, 409–422 (1981).
8. McCall, G. J. H. *Geol. Surv. Kenya Rep. No. 65* (1964).
9. Vearncombe, J. G. *J. Afr. Earth Sci.* **1**, 133–144 (1983).
10. Meinhold, K. D. *Revue Géogr. phys. Géol. dyn.* **21**, 395–401 (1979).
11. Ries, A. C., Shackleton, R. M., Graham, R. H. & Fitches, W. R. *J. geol. Soc. Lond.* **140**, 75–95 (1983).
12. Pegram, W. J., Sands, J. W. & Hodges, K. V. *Geol. Soc. Am. abstr. Prog.* **12**, 497 (1980).
13. Stacey, J. S. & Stoesser, D. B. *Contr. Miner. Petrol.* **84**, 91–105 (1983).
14. Gillespie, J. G. & Dixon, T. H. *Precamb. Res.* **20**, 63–77 (1983).
15. Hashad, A. H., Sayyah, J. A., Al-Kholy, S. B. & Youseff, A. *Egypt. J. Geol.* **16**, 269–281 (1972).
16. Davidson, J. P. *Nature* **306**, 253–256 (1983).
17. Vail, J. R. *Sudan Dept. Geol. Miner. Resour. Bull. No. 23* (1973).
18. Vail, J. R. *Bull. Inst. appl. Geol. Jeddah* **3**, 97–109 (1979).
19. El Shazley, E. M., Hashad, A. H., Sayyah, J. A. & Bassayuni, F. A. *Egypt. J. Geol.* **17**, 1–18 (1973).
20. Almond, D. C. *Precamb. Res.* **13**, 43–62 (1980).
21. Vail, J. R. *Proc. R. Soc. A350*, 127–141 (1976).
22. Dixon, J. H. *Precamb. Res.* **14**, 119–133 (1981).
23. Abdel Monem, A. A. & Hurley, P. M. *Bull. Inst. appl. Geol. Jeddah* **3**, 165–170 (1979).
24. Bertrand, J. M. L. & Caby, R. *Geol. Rdsch.* **67**, 357–388 (1978).
25. Gaudette, H. E., Hurley, P. M., Fairbairn, H. W. & Lajmi, T. *21st a. prog. Rep.* (Geochronology Laboratory, MIT, 1976).
26. Gabert, G. & Wendt, I. *Geol. Jber.* **B11**, 3–55 (1974).
27. Allègre, C. J. & Ben Othman, D. *Nature* **286**, 335–342 (1980).
28. Davies, G. R. thesis, Open Univ. (1984).
29. Hawkesworth, C. J. & Rogers, G. *EOS* **64**, 330 (1983).
30. Hawkesworth, C. J., Van Calsteren, P. & Menzies, M. A. *J. geol. Soc. Lond.* (in the press).
31. Hawkesworth, C. J., Hammill, M., Gledhill, A. R., Van Calsteren, P. & Rogers, G. *Earth planet. Sci. Lett.* **58**, 240–254 (1982).

Effects of acidification and complexation from radiolytic reactions on leach rates of SYNROC C and nuclear waste glass

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Previous work¹ has shown that γ -radiation induced enhancement of leach rates from high-level radioactive waste (HLW) solids can be attributed to radiolytic acidification of the aqueous solution and to radiolytic formation of organic complexing species, for example, $(\text{COOH})_2$, HCOOH . Barkatt *et al.*¹ compared the leach resistance of three HLW glasses and SYNROC D (specifically designed for incorporation of US defence wastes²) and found that, at low pH and with complexing agents, higher alumina contents in the waste form gave large enhancement of leach rates. Specifically, in acid solution, the elemental leach rates for SYNROC D were increased relative to the glasses by factors between 20 and 1,200. The formulation of SYNROC C, designed by Ringwood² for incorporation of reprocessed waste from commercial power reactors, was not tested in these conditions. We report here that SYNROC C is considerably more resistant than the standard HLW glass PNL 76–68 to acid attack and to possible complexation reactions between 90 and 150 °C, despite an alumina content in the formulation that is much higher than that for PNL 76–68. The Al content of SYNROC C is much lower than that of SYNROC D (Table 1) and it is largely present in the hollandite phase after hot pressing. No aluminosilicate phases are formed in SYNROC C and, for this reason, the pH dependence is much less marked. These results are significant in assessing the effects of changes induced by the ionizing radiation to which the HLW solid and solution will be exposed during geological isolation.

Table 1 Compositions (wt%) of SYNROC and waste glass PNL 76-68

	SYNROC C (batch 160)	SYNROC D (S21S18)	PNL 76-68
TiO ₂	56.0*	21.9	3.0
ZrO ₂	11.9*	5.8	1.9
Al ₂ O ₃	5.4*	18.9	—
SiO ₂	<0.1*	6.7	38.3*
CaO	13.5*	6.6	2.5*
BaO	5.7*	1.2	0.5*
Fe ₂ O ₃	1.1	23.0	10.3
B ₂ O ₃	—	—	9.1*
SrO	0.7*	0.2	0.5*
Cs ₂ O	0.7*	0.2	1.2*
Na ₂ O	—	3.2	12.8
Rare earths and others	5.0	12.3	19.9

* Measured values, otherwise nominal compositions are given.

Ringwood has proposed several different formulations of SYNROC to accommodate various types of high-level nuclear waste². The two formulations that have received most attention are SYNROC C and SYNROC D. Commercial and defence wastes differ in composition and the corresponding SYNROCs also vary considerably in mineralogies and properties^{3,4}. The compositions, on an oxide basis, of both SYNROCs and the standard HLW glass PNL 76-68, supplied by the Materials Characterization Center (MCC) at Pacific Northwest Laboratory (PNL)⁵, are given in Table 1. SYNROC C can immobilize >20 wt% commercial wastes and has four major phases of perovskite (CaTiO₃), hollandite (Ba (Al, Ti³⁺)₂Ti₆O₁₆), zirconolite (CaZrTi₂O₇) and rutile TiO₂ with some minor metal alloy phases³. SYNROC D, however, designed to accommodate up to 65% defence wastes, has major phases of hollandite, zirconolite and perovskite but also an aluminosilicate nepheline (NaAlSi₃O₈) and a spinel titanate phase ((Fe, Ni, Mn)(Al, Fe)₂O₄-(Fe, Ni, Mn)₂TiO₄)⁴. The last two phases normally comprise about two-thirds of SYNROC D on a volume basis. The high alumina content of SYNROC D arises, not from SYNROC D additives, but from the waste itself.

Barkatt *et al.*¹, in examining the effect of γ -radiolysis on HLW glasses and SYNROC D, reported HNO₃, HCOOH and (COOH)₂ at concentrations of 78, 46 and 30 μ mol dm⁻³ after 3 days irradiation with a ¹³⁷Cs source (8.6×10^4 rad h⁻¹). Although it is recognized¹ that a very low pH is unlikely to be found in a high-level waste repository (owing to buffering by rock minerals), the local effect of acidification near the waste surface as well as the effect of organic complexing agents in solution may be important. We have tested both effects in a comparison of SYNROC C and PNL 76-68.

The SYNROC C used in our experiments was prepared by the standard oxide-route method⁶. This entailed mixing the

precursor oxides or carbonates with a simulated waste solution containing a shortened version of the reference US commercial waste PW-4b⁶. The waste loading was 10 wt% on an oxide basis. After calcination at 1,100 °C in an Ar/3% H₂ atmosphere, 2 wt% Ti powder was added and the blended powder hot-pressed at 1,250 °C for 3 h. The test specimens were 10 mm diameter by 2-mm thick discs of SYNROC C and PNL 76-68 prepared according to the method recommended by the MCC⁵. The flat faces of these discs were ground to a 240-300 grit finish before leaching.

Table 2 compares the leach rates of SYNROC C and PNL 76-68 in static test conditions (MCC-1) at 90 °C for 7 days. The leachants were pH 1.8, 4.2, 7.0 and 9.0 buffer solutions. The results clearly show that, in all test conditions, the leach resistance of SYNROC C is superior to that of borosilicate glass PNL 76-68. Furthermore, the leach rate of SYNROC C is less susceptible than glass to changes in pH. The difference in the leach rate of the major SYNROC matrix elements, titanium and zirconium, compared with that of the major borosilicate glass matrix elements, silicon and boron, is particularly striking. Although the leach rate of Ti from SYNROC C increases by a factor of >250 when the pH is lowered from 7 to 1.8, it is still 200 times lower than that of silicon from glass. The method of reporting results as a ratio of leach rates at two different pH values, used by Barkatt *et al.*¹, tends to obscure such information.

Campbell *et al.*⁷ have measured the leach rates of SYNROC D in deionized water in identical test conditions (MCC-1). Table 3 shows that SYNROC C is clearly more resistant to leaching than SYNROC D and PNL 76-68. The differences in leach rates of the two types of SYNROC are clearly indicated in the results for Al, Ca and Sr. Table 3 also indicates that the leach rate of Si from SYNROC D is quite high. These facts support the contention^{1,2} that it is the aluminosilicate phase in SYNROC D, known to exhibit a sharp dependence of dissolution on pH, that is leachable. Conversely, our results show that the absence of aluminosilicate phases in SYNROC C lead to much enhanced resistance to leaching.

Table 4 compares leach rates of SYNROC C and PNL 76-68 in more extreme conditions. Hydrothermal leaching at 150 °C in Teflon-lined Parr autoclaves was used to simulate conditions that could occur if shorter storage times were used before disposal. We have used an acidic solution of nitric acid (0.16 mol dm⁻³), formic acid (0.37 mol dm⁻³) and oxalic acid (0.01 mol dm⁻³) in the proportions 2:1:1 by volume to test the effect of organic acid complexation as well as acidification (pH was adjusted to 1.5 with concentrated ammonia solution). The discs were leached in 10 cm³ of the acid mixture for 1 day; the leachant was then replaced and leaching continued for a further 5 days. Table 4 shows the leach rate for the first day and the average for the entire 6 days. After leaching of the glass, a gel layer had formed on the Teflon container walls and a sediment settled on the bottom of the container. These solids were

Table 2 Leach rate (g m⁻² day⁻¹) of borosilicate glass (PNL 76-68) and SYNROC C (batch 160) as a function of pH (MCC-1 test conditions at 90 °C for 7 days)

	Buffer pH = 1.8		Buffer pH = 4.2		Buffer pH = 7.0		Buffer pH = 9.0	
	Glass	SYNROC	Glass	SYNROC	Glass	SYNROC	Glass	SYNROC
Final pH	1.89	1.80	4.35	4.20	7.2	6.9	9.41	9.0
Element								
Cs	8.8	0.88	0.77	0.41	0.57	0.31	1.9	0.39
Sr	6.7	0.11	0.63	0.015	0.39	0.009	0.30	0.009
Ca	4.4	0.10	0.39	0.032	0.25	0.020	0.16	0.021
Ba		0.094		0.030		0.010		0.019
Al		0.11		0.03		0.03		0.05
Si/Ti*	9.7	0.05	0.93	<0.0002	0.54	<0.0002	1.8	<0.0002
B/Zr*	10.0	0.008	0.84	<0.001	0.68	<0.001	2.4	<0.001
Mass	8.0	0.13	0.51	0.07	0.36	0.03	0.81	0.04

* Glass results are for Si and B; SYNROC results are for Ti and Zr.

Table 3 Leach rates ($\text{g m}^{-2} \text{day}^{-1}$) of SYNROC and borosilicate waste glass in deionized water (MCC-1 test conditions at 90 °C for 7 days)

	Glass PNL 76-68	SYNROC C batch 160	SYNROC D* batch S21S18
Final pH	9.40	5.3	6.0
Element			
Cs	1.8	0.39	0.74
Sr	0.42	0.011	1.17
Ca	0.16	0.017	0.41
Ba	NA	0.021	0.05
Al	NA	0.03	0.47
Si	1.9	NA	2.29
Ti	NA	<0.0002	<0.0003
B	2.4	NA	NA
Zr	NA	<0.001	<0.001
Mass	0.82	0.01	0.30

NA, Not applicable.

* From ref. 7, Cs was presynthesized in hollandite.

Table 4 Average leach rate ($\text{g m}^{-2} \text{day}^{-1}$) of borosilicate glass (PNL 76-68) and SYNROC C (batch 114) in hydrothermal conditions (150 °C) at pH 1.5 in acid mixture (nitric, formic, oxalic)

Wasteform	Glass		SYNROC C	
Total leach time (days)	1	6	1	6
Final pH	2.5	2.6	2.1	2.2
Element				
Cs	43.2 (72.9)	17.1 (27.1)	13.0	4.8
Sr	45.0 (64.7)	15.9 (22.8)	2.5	1.3
Ca	20.1 (*)	8.1 (*)	2.7	1.4
Ba	NA	NA	0.55	0.23
Si	35.6 (74.0)	15.5 (26.1)	NA	NA
Na	54.4 (*)	18.8 (*)	NA	NA
Mass	80.5	28.4	2.5	0.8

NA, Not applicable. Numbers in parentheses include contributions due to sediments.

* Interference precluded analysis.

dissolved and their considerable contribution to the leach rate was determined as shown by the numbers in parentheses in Table 4. The results show clearly that in these extreme conditions, SYNROC C has superior leach resistance. When compared with results at pH 1.8 (Table 1), it is seen that the leach rates of both glass and SYNROC are enhanced by the combination of lower pH, higher temperature and organic acids by a factor generally <10.

The leach rates we have reported are based on relatively short-term tests (7 days) and relatively low surface area/solution volume ratios. They therefore represent a comparison of relative reactivity towards acid attack and organic acid complexation in initial leaching. The long-term effects of, for instance, solution saturation, reprecipitation and recrystallization have not been considered but some comments on Cs, the most leachable radwaste element, are relevant. Table 4 shows that, in acidic hydrothermal conditions, the leach rate of glass and SYNROC C decreases with time. Glass leaches more or less congruently, but caesium is the most leachable element from SYNROC C. The relatively high initial leach rate of caesium from SYNROC occurs because a small amount (<5%) is incorporated not in the hollandite phase but in intergranular films⁸. The high initial rate is caused by the leaching of this loosely bound caesium. Preferential leaching during the first few days depletes the near-surface region of the intergranular regions and as the thickness of the leached layer increases, so the rate of dissolution decreases. Solomah and Zumwalt⁹ found that at 25 °C the caesium leach rate fell from $0.07 \text{ g m}^{-2} \text{day}^{-1}$ after 3 days to $0.0004 \text{ g m}^{-2} \text{day}^{-1}$ after 100 days. Similarly, Reeve *et al.*⁶ found that the leach rate at 100 °C fell from $0.4 \text{ g m}^{-2} \text{day}^{-1}$ after 1 day to $0.002 \text{ g m}^{-2} \text{day}^{-1}$ after 70 days. The caesium leach rate can be improved further⁶ if the SYNROC precursor is prepared

by hydrolysis of titanium and zirconium alkoxides following the SANDIA method¹⁰. The leach rate of Cs from glass is constant when the leachant replacement rate is high⁶. In static conditions the average leach rate decreases by a factor of about three over the first 100 days¹¹ but, like SYNROC C, the instantaneous leach rate of Cs after 6 months is not measurable^{6,11}.

It is clear, then, that acidification and complexation effects resulting from γ -radiolytic reactions may be important in assessing different HLW forms. The incorporation of alumina as an aluminosilicate in crystalline materials or as a network-former in glassy materials could result in a large increase in leachability in irradiated aqueous media. SYNROC C, in which the alumina is incorporated into the hollandite phase, shows a much smaller increase than PNL 76-68 or SYNROC D in leachability in conditions expected in the first phase after disposal.

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1. Barkatt, A., Barkatt, A. & Sousanpour, W. *Nature* **300**, 339-341 (1982).
2. Ringwood, A. E. *et al. Nucl. chem. Waste Manag.* **2**, 287-305 (1981).
3. Cousens, D. R. *et al. Scientific Basis for Radioactive Waste Management* Vol. 5 (ed. Lutze, W.) 310-317 (Elsevier, New York, 1982).
4. Campbell, J. *et al. High Level Waste Technical Review*, Atlanta (UCRL-85913, 1981).
5. *Nuclear Waste Materials Handbook—Test Methods (DOE/TIC-11400, Rev 1 Materials Characterization Center, Pacific Northwest Laboratory, 1983).*
6. Reeve, K. D., Levins, D. M., Ramm, E. J. & Woolfrey, J. L. *Proc. int. Symp. on Conditioning of Radioactive Wastes for Storage and Disposal*, 375-390 (IAEA, Vienna, 1983).
7. Campbell, J. *et al. Properties of SYNROC D Nuclear Waste Form: A State-of-the-Art Review* (UCRL-53240, 1982).
8. Cooper, J. *et al. The Incorporation and Retention of Caesium in SYNROC C* (Griffith University).
9. Solomah, A. G. & Zumwalt, L. R. *Nucl. chem. Waste Manag.* **3**, 111-115 (1982).
10. Dosch, R. G. & Lynch, A. E. *Solution Chemistry in SYNROC Preparation* (SAND 80-2375, 1980).
11. Strachan, D. M. *Results from Long-term Use of the Static Leach Test Method (MCC-1) (PNL-SA-10762, 1982).*

Evidence of two temperate episodes in late Pleistocene deposits at Marsworth, UK

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In the British Quaternary, two post-Cromerian interglacials, the Hoxnian and the Ipswichian, are recognized¹. Evidence of additional interglacials in this interval is widely accepted in the oceanic record of Quaternary events², and the possibility that at least one additional interglacial of this age is represented in Britain has been discussed³⁻⁵. However, in the absence of datable interglacial deposits which are seen to overlie one another, the issue has remained controversial. We describe here deposits at Marsworth, UK (Fig. 1) where there is evidence of two temperate episodes, and of intervening periglacial conditions. Stratigraphical superposition is established beyond any reasonable doubt. The later deposit relates to the temperate woodland stage of the Ipswichian Interglacial. Dating of the earlier temperate material by the ²³⁰Th/²³⁴U disequilibrium method indicates an interglacial episode not previously established in the British Quaternary.

Table 1 Mammal faunas of the Marsworth channels

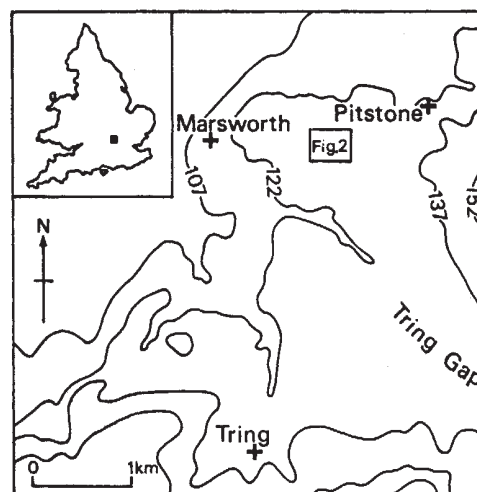
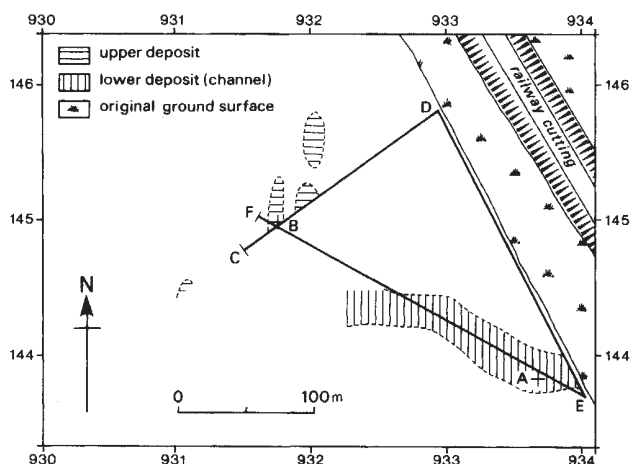
Upper channel	Elephant (indeterminate) <i>Dicerorhinus hemitoechus</i> (Falconer) <i>Hippopotamus amphibius</i> Linné <i>Megaceros giganteus</i> (Blumenbach) <i>Cervus elaphus</i> Linné <i>Bison priscus</i> Bojanus <i>Arvicola terrestris</i> Linné <i>Microtus</i> sp.
Lower channel	<i>Canis lupus</i> Linné <i>Ursus arctos</i> Linné <i>Panthera leo</i> Linné <i>Mammuthus primigenius</i> Blumenbach <i>Equus ferus</i> Boddaert <i>Equus hydruntinus</i> Regalia Cervid (indeterminate) Bovini (indeterminate) <i>Microtus oeconomus</i> (Pallas)

At the base of the succession at A (Fig. 2), a channel up to 35 m wide in the surface of the chalk (lower channel) contains sediments of a shallow stream. The channel-fill includes gravelly sands below, passing up into organic mud. The organic mud is confined to the area indicated in section E-F (Fig. 3), but the gravelly sands have been traced continuously in this section. The organic muds and the gravelly sands are overlain by chalky muds full of small chalk pellets. Lenses of water-laid sand and fine gravel are also present. These largely colluvial deposits, up to 4 m thick, are found within and beyond the lower channel and are overlain by a coarser head of chalk and flint debris. This Coombe Rock, 2–3 m thick and involuted in its upper part, can be traced across the site of the lower channel and widely beyond it.

At B (Fig. 2), a separate channel (upper channel), 8 m wide, was present in the surface of the Coombe Rock, truncating the involutions. It contained finely stratified sandy gravel, and marl. There were several small pond-like depressions containing similar deposits nearby.

The lower channel-fill contains abundant fossil material, including molluscs, ostracods, beetles, vertebrates and pollen and plant macrofossils. Travertine is present in clasts up to 0.8 m long, and bears occasional leaf impressions of temperate woodland species, including *Acer*. It also contains Mollusca of temperate woodland habitats, including *Azeca goodalli*, *Discus rotundatus* and *Clausilia bidentata*. The upper channel contained abundant mammalian bone, but no other fossil material.

In the mud of the lower channel-fill, herbs dominate the pollen and macrofossil flora throughout. Gramineae are best represented in the pollen record, with subsidiary amounts of Cyperaceae and many grassland herbs. The small quantities of tree and shrub pollen consist mainly of *Pinus* with occasional *Picea*, *Abies*, *Betula*, *Quercus*, *Ulmus*, *Alnus*, *Salix*, *Juniperus* and *Ribes*. Aquatic pollen is present (*Potamogeton*, *Hippuris* and *Butomus*) and late in the sequence the spore flora includes *Lycopodium clavatum* and *Selaginella selaginoides*. The Mollusca are predominantly undemanding in their habitat requirements (for example, *Trichia hispida*, *Arianta arbustorum*). In the lower part of the sequence, marsh and aquatic species occur, including *Oxyloma pfeifferi* and *Sphaerium corneum*. In the upper part of the sequence they are replaced by grassland species such as

**Fig. 1** Map showing the location of Marsworth.**Fig. 2** Pleistocene fossiliferous deposits in Pitstone no. 3 quarry, Marsworth, showing alignment of sections illustrated in Fig. 3. National Grid coordinates are given at the margin (100 km square SP).

Pupilla muscorum, together with many fragments of *A. goodalli* and *C. bidentata*, possibly derived from earlier sediments or soils. The lower part of the sequence yields an ostracod fauna of limited species diversity, dominated by *Prionocypris serrata*, and including *Ilyocypris bradyi*, *Candona neglecta* and *Potamocypris* spp.

Eighty species of Coleoptera are present and suggest a temperate marshy grassland habitat dominated by *Glyceria maxima*, the foodplant of *Donacea semicuprea* and *Notaris acidulus*, which are both common. The abundance of dung beetles suggests that the grassland was grazed, and the small carnivorous beetle *Anotylus gibbulus*, which is also common, is probably associated with the dung of large herbivores. The central and southern European weevil *Stomodes gyroscollis*, which lives on

Table 2 Uranium series disequilibrium ratios and ages of travertines obtained from the lower channel at Marsworth

Harwell sample no.	Acid-insoluble residue	Uranium content (p.p.m.)	$^{234}\text{U}/^{238}\text{U}$	Activity ratios $^{230}\text{Th}/^{234}\text{U}$	$^{230}\text{Th}/^{232}\text{Th}$	Age (kyr)
HUTH 1359	Nil	0.14 ± 0.02	0.81 ± 0.16	1.910 ± 0.347	5	UD
HUTH 1360	Nil	0.12 ± 0.02	4.41 ± 0.83	1.033 ± 0.107	17	UD
HUTH 1539	Nil	0.06 ± 0.01	2.16 ± 0.19	0.880 ± 0.065	12	171^{+28}_{-24}
HUTH 2215	Nil	0.08 ± 0.01	1.48 ± 0.13	0.833 ± 0.071	15	165^{+34}_{-27}
HUTH 2237	Nil	0.10 ± 0.01	1.55 ± 0.08	0.795 ± 0.041	37	149^{+27}_{-14}

UD, Undateable. * All errors quoted are $\pm \sigma$ statistical error only.

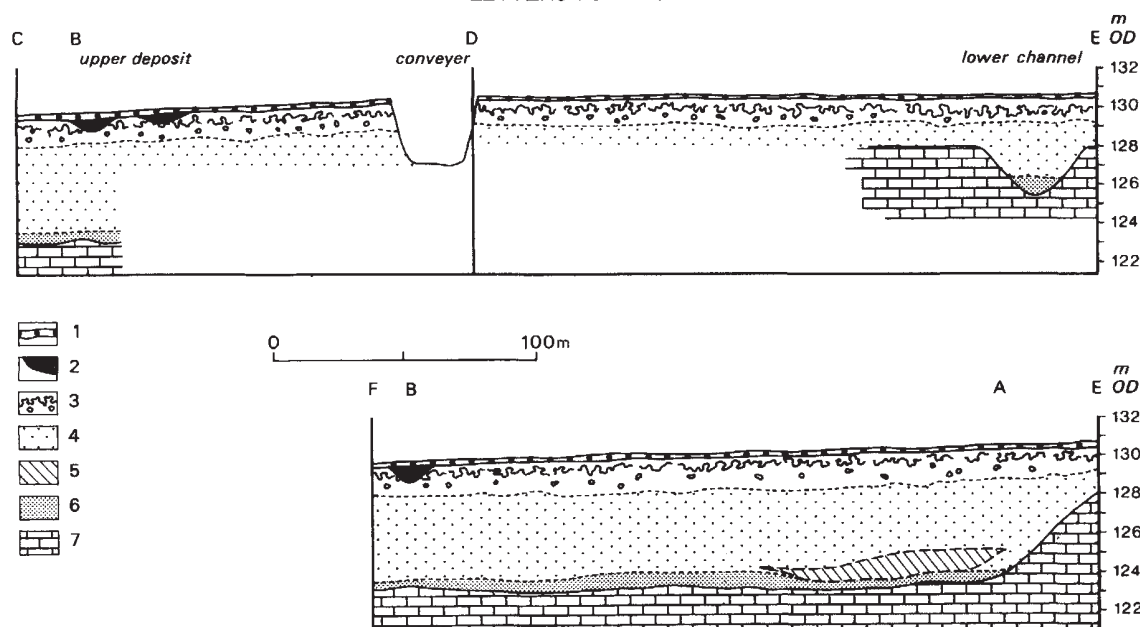


Fig. 3 Sections in Pitstone no. 3 quarry, Marsworth. 1, Topsoil; 2, upper deposit; 3, Coombe Rock with involutions; 4, chalk muds with waterlaid sand and fine gravel; 5, organic muds; 6, fossiliferous gravelly sand; 7, Lower Chalk.

various species of clover, is present. Late in the sequence, an increase of the less thermophilic species, including *Pyncoglypta lurida*, *Arpedium brachypterum* and *Olophrum fuscum*, occurs. The mammal fauna (Table 1) is an assemblage recorded at several sites in Britain^{6,7}. These *Mammuthus-Equus* faunas are distinguished by a large form of lion, *Panthera leo*, and a small form of wolf, *Canis lupus*. At Marsworth, the fauna includes *Microtus oeconomus* and the extinct equid, *Equus hydruntinus*.

The fossils in the lower channel-fill indicate a shallow spring-fed stream with backwater slacks, in a local context of marshy grassland. The stream was subsequently replaced by *Carex*-dominated swampy depressions, and these were finally infilled by colluvial debris. The regional vegetation was predominantly grassland throughout the period of record. A climate similar to that of northern England today is represented, with indications in the travertine of earlier warmer conditions, and in the deposits themselves and the fossils contained therein, of slight cooling during sedimentation.

Travertine from the lower channel was dated by the ²³⁰Th/²³⁴U disequilibrium method (Table 2). Samples HUTH 1539, HUTH 2215 and HUTH 2237 yielded analytical results indicating a good standard of reliability⁸⁻¹⁰, and together suggest a range of travertine deposition ages between 140 and 170 kyr.

In the upper channel, the mammal fauna (Table 1) is a characteristic temperate woodland assemblage, known from many other British sites and apparently restricted to the Ipswichian Interglacial^{6,7,11}. Speleothem enclosing a similar fauna in Yorkshire has yielded uranium series dates around 120 kyr BP (ref. 12). Stratigraphical relationships at Marsworth (Figs 2, 3) show that the upper channel-fill overlies the lower. The coombe deposits separating the channel-fills suggest an intervening period of severe periglacial conditions.

Dating of the travertine in the lower channel indicates a pre-Ipswichian age, but one too young to fall within the Hoxnian. In addition, the mammal fauna in the lower channel is unlike faunas of Hoxnian age. *Mammuthus-Equus* faunas occur in middle Devensian contexts^{6,7}, but differ in composition from that at Marsworth. Sites having *Mammuthus-Equus* assemblages comparable with Marsworth have generally been assigned to the late Ipswichian^{6,7}. The Marsworth evidence demonstrates that a pre-Ipswichian age is possible for such assemblages^{13,14}.

The beetle *A. gibbulus*, which is common at Marsworth, has not been found in faunas of firmly established Hoxnian or

Ipswichian age. It occurs very rarely in middle Devensian deposits, but in assemblages unlike that from Marsworth. It is relatively common (G.R.C., unpublished data) in interglacial sediments at Stoke Goldington in the Great Ouse valley, at Aveley in the Lower Thames Valley, and at Stanton Harcourt in the Upper Thames Valley. At none of these sites are the total fossil assemblages comparable with examples of Hoxnian or Ipswichian age.

The Marsworth pollen shows no clear resemblance to established floras of either interglacial or interstadial status. In the Hoxnian and the Ipswichian, *Picea* was scarce, and while *Abies* was present towards the end of the Hoxnian, it was rare or lacking in the Ipswichian¹⁵. The middle Devensian, Upton Warren Interstadial has a flora with temperate, boreal and arctic-alpine components, with grassland as the major vegetation¹⁶. In the early Devensian forested interstadials, *Betula*, *Pinus* and *Picea* are the usual tree components and *Abies* is unknown. At High Lodge, in what may be a Wolstonian interstadial context¹⁵, open coniferous woodland occurs with *Betula*, *Pinus*, *Picea* and *Alnus*, Gramineae and open-ground herbs. Despite the variety of these interstadial floras, they all differ from Marsworth.

We conclude that the travertine in the lower channel represents a previously unrecorded interglacial, intermediate in age between the Hoxnian and the Ipswichian. We note in particular that it preserves temperate woodland species. The sediments of the lower channel, which enclose the travertine, indicate a grassland environment. We note the absence of obligate cold species in the contained fossil assemblages, and conclude that the sediments probably represent conditions succeeding those recorded in the travertine, but within the same temperate episode. It is possible, however, that the sediments denote an additional pre-Ipswichian temperate phase of interstadial status.

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1. Mitchell, G. F., Penny, L. F., Shotton, F. W. & West, R. G. *Geol. Soc. Lond. Spec. Rep.*, 4 (1973).
2. Shackleton, N. J. in *British Quaternary Studies* (Oxford University Press, 1977).
3. Sutcliffe, A. J. *Quat. Newslett.* 18, 1-7 (1976).
4. Bowen, D. Q. *Quaternary Geology* (Pergamon, Oxford, 1978).
5. Shotton, F. W. in *Quaternary Glaciations in the Northern Hemisphere* IGCP Project 73/1/24 Report, 9 (1983).
6. Stuart, A. J. *Phil. Trans. R. Soc. B276*, 221-250 (1976).

7. Stuart, A. J. *Pleistocene Vertebrates in the British Isles* (Longman, London, 1982).
8. Gascoyne, M., Schwarcz, H. P. & Ford, D. C. *Trans. Br. Cave Res. S.* 5, 91–111 (1978).
9. Schwarcz, H. P. *Archaeometry* 22, 3–24 (1980).
10. Ivanovich, M. in *Uranium Series Disequilibrium* (eds Ivanovich, M. & Harmon, R. S.) (Oxford University Press, 1982).
11. Sutcliffe, A. J. *Trans. Torquay nat. Hist. Soc.* 13, 1–26 (1960).
12. Gascoyne, M., Currant, A. P. & Lord, T. C. *Nature* 294, 652–654 (1981).
13. Hinton, M. A. C. *Monograph of the Voles and Lemmings (Microtinae) Living and Extinct* (British Museum (Natural History), London, 1926).
14. Sutcliffe, A. J. & Kowalski, K. *Bull. Br. Mus. nat. Hist. (Geol.)* 27, 33–147 (1976).
15. West, R. G. *New Phytol.* 85, 571–622 (1980).
16. Coope, G. R., Shotton, F. W. & Strachan, I. *Phil. Trans. R. Soc. B* 244, 379–421 (1961).

A real-time dynamical forecast of ocean synoptic/mesoscale eddies

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Energetic eddy currents are the oceanic analogue of atmospheric weather and have time scales of days to weeks and space scales of tens to hundreds of kilometres¹. We have predicted their evolution in time by internal dynamical processes. For the forecasts we transferred initialization data from ship- to shore-based computers, during two 2-week periods in June and July of 1983 from an approximately (150 km)² region in the deep north-east Pacific off California. This is, to the best of our knowledge, the first such real-time forecast at sea. The results were substantially successful; the dynamics predicted a prominent zonal jet absent in the initial flow. Also, the model forecast revealed a strong eddy merger event otherwise obscured in the data. Prospects are encouraging for future forecasting, which should have important implications for research and practical operations at sea².

The efficient description and prediction of the flow field in the ocean is necessary because of the difficulty and cost of direct observations due both to the opacity and vastness of the sea and to the natural scales of the phenomena. Modern predictive methodology is developing, guided by related experience in meteorology, astronomy and engineering, and based on optimal estimation procedures. Key issues are data assimilation in dynamical models³, which involves the regular and synoptic gridding (analysis) of gappy multicomponent data sets, and the understanding of initialization and verification procedures. Our concept invokes a descriptive predictive system^{4,5} consisting of an observational network, a dynamical model and a statistical model. The statistical model is an anisotropic mixed space-time objective analysis scheme which depends on east, west and time lags⁶; it is used for initialization and verification analyses for dynamical forecasts. The dynamical model is a baroclinic quasigeostrophic model which interrelates the flow, density and pressure fields. The open (water–water) boundary conditions, which allow the utilization of the model in arbitrarily located regions, require the specification of the normal flow everywhere around the region and the vorticity (horizontal gradients of horizontal flow components) on inflow. The model has been documented, calibrated, and applied to hindcasts of the POLY-MODE synoptic/dynamics experiment data⁵. With adequate computational resolution and accurate boundary conditions, the model can forecast its interior domain for months, with a normalized r.m.s. error of only a few per cent⁷.

Our 'test block' of ocean, located in the California Current System, is centred at 38°15' N and 126° W, with a mean depth of 4,200 m. Although close to topographic features, the bottom slope is generally only 0(10⁻²) or less in the region. Three surveys in 1982 found highly variable flow consisting of current filaments

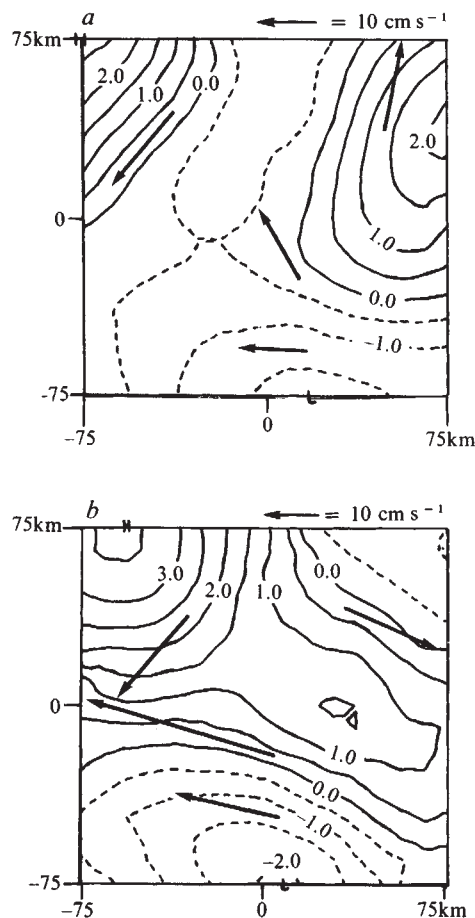


Fig. 1 *a*, Streamfunction (pressure field) at 150-m depth in the main thermocline for Julian day 5506 (20 June 1983), used as the initial condition for forecast and forecast experiment. The centre of the domain is at 38°15' N, 126° W. The ordinate is rotated 10° west of north. The scale of the streamfunction is 5,000 m² s⁻¹. Arrows indicate flow speed and direction. The contour interval is 0.5. *b*, As for *a*, Julian day 5520 (4 July 1983) used as verification for both the forecast and forecast experiment, also used in the linear interpolation of boundary conditions for the forecast experiment.

intermingled with cyclonic and anticyclonic eddies. Substantial changes in the flow system can occur within a week⁸. These cruises were carried out to provide the requisite kinematical background for the forecast reported here. In 1983, three hydrographic surveys each of a week duration were obtained 2 weeks apart, to provide two sets of initialization and verification data. Each hydrographic survey consisted of approximately 90 XBT (expendable bathythermograph) measurements of the vertical temperature profile interspersed with 10 CTD (conductivity–temperature–depth) measurements of both the temperature and salinity profiles. Nominal station spacing along the ship track was ~10 km. Temperature and salinity are well correlated in this region so the mean temperature–salinity relationship was used together with the XBT measurements to obtain density profiles and geostrophic currents (dynamic height). Although we plan direct current measurements in future work, the thermocline flow pattern is not sensitive to reasonable assumptions about a geostrophic reference level. Analyses were carried out for each central day by combining data and mapping with a time-independent objective analysis scheme. The first and second analyses are shown in Fig. 1*a, b*.

Originally (Fig. 1*a*) the flow in the centre of the region was weak with a strong anticyclonic eddy in the north-east corner, a southwestward current, part of an anticyclonic eddy, in the

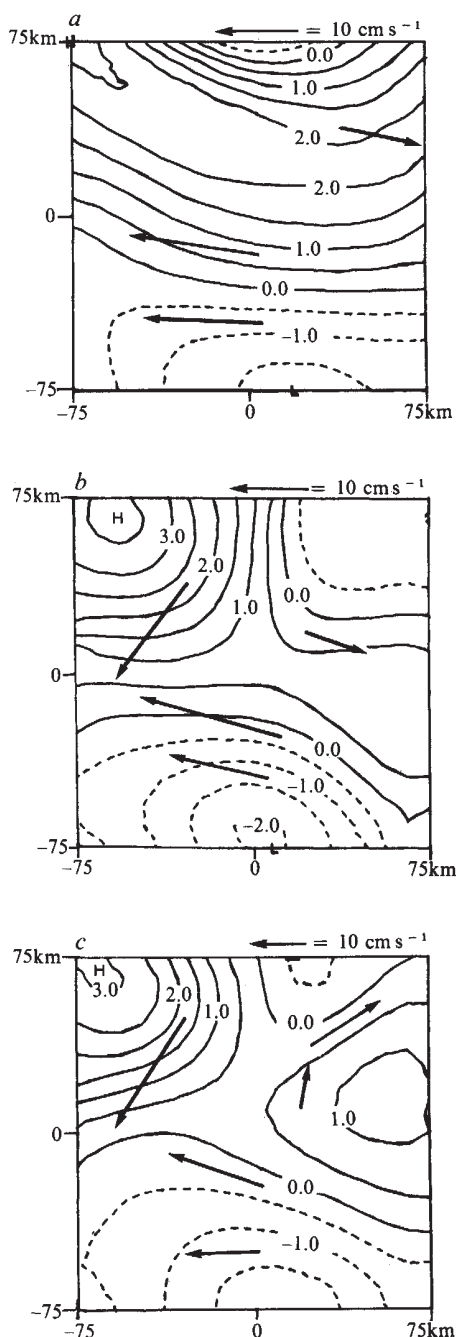


Fig. 2 *a*, Forecast streamfunction at 150-m depth for Julian day 5520 (a 2-week forecast), using persistent boundary conditions (those of Julian day 5506). *b*, Forecast experiment streamfunction at 150 m for Julian day 5520 (a 2-week forecast), using linearly-interpolated boundary conditions (between Julian day 5506 and 5520). *c*, As for *b*, at Julian day 5513 (the forecast after 1 week).

north-west and a segment of cyclonic flow in the southern boundary. Two weeks later (Fig. 1*b*) the flow had dramatically changed; the centre was dominated by a strong zonal jet, the anticyclones in the north-west and north-east had apparently merged, and the southern cyclone intensified. After another 2 weeks (not shown), the anticyclonic eddy was further enlarged and the jet, now entering from the eastern edge and oriented towards the south-west, filled the entire central and southern region.

For the dynamical model forecast runs, physical resolution and computational accuracy considerations led to a time step of 3 h, 9 km horizontal resolution and six vertical levels

throughout the water column. The hydrographic measurements directly provide only velocity shear in the thermocline, but to initialize the model we need absolute velocity in the thermocline and an estimate of the velocity throughout the water column including the deep water. To estimate the shear below the bottom of the XBT casts, we projected the thermocline flow onto the first baroclinic dynamical mode. The flow in the deep water was then taken to be composed of only the barotropic and first baroclinic modes, with an amplitude ratio determined by numerical experiments. Thus determined, the deep flow estimate provides a velocity at 500 m, which was used as a reference value for the thermocline shear. This choice of deep structure, and the ability to estimate the first mode structure from thermocline measurements only, are supported by our related studies with empirical orthogonal functions. After trying different mode ratio assumptions in real time, a barotropic mode was added which had 75% of the energy of the first baroclinic mode and opposed it below the thermocline. This initial mode ratio resulted in the least rapid change of the amplitude ratio of the barotropic to first baroclinic mode at the start of the forecast. The forecasted thermocline flow patterns were in any case found to be insensitive to the assumptions about the mode ratios.

Real-time forecasts were performed by initializing the dynamical model with the analysed data and by simply persisting the initial boundary conditions for the duration of the forecast. Later we performed a dynamical 'forecast experiment' with boundary conditions linearly interpolated in time between the initialization and verification analyses. The forecasts are shown in Fig. 2(*a*, *b*) for the cases of persisted (interpolated) boundary conditions.

During the forecasts the anticyclone in the north-west corner moved into the region, merged with the anticyclone in the north-east corner, which then weakened and disappeared; the small closed contour in the eastern central region of Fig. 1*b* is the remnant of the original north-east corner anticyclone. The southern cyclone enlarged, strengthened and also contributed to the zonal jet. This dynamical behaviour occurred in both the forecast and the forecast experiment and the zonal jet is seen prominently in Figs 1*b*, 2*a*, *b*. The (incorrect) persisted boundary conditions of the forecast alter the flow pattern at the edges and ultimately suppress the north-west corner eddy. However, the forecast experiment with interpolated boundary conditions represents very well the flow pattern and current strengths everywhere. This indicates that the dynamical model can 'project' good resolution boundary data into a rather large domain. Results for the second period were similar.

To illustrate the power of the model as an interpretative physical tool, we show in Fig. 2*c* the flow field a week after the start of the forecast experiment. Without dynamical model interpolation it is impossible to relate unambiguously the synoptic realizations of Fig. 1 separated by only 2 weeks. Figure 2*c* shows the anticyclone originally in the north-east weakened to half its original strength; the jet has formed and the southern cyclone is present. Elucidation of the nonlinear processes inherent in the eddy merger and jet production shown in Fig. 2 will be a contribution to eddy dynamics.

The success of our real-time dynamical forecast is encouraging for the prospects of practical real-time ocean forecasting. The substantial improvement of the dynamical forecast with interpolated boundary condition directs our research now towards: (1) statistical forecasting of the boundary conditions for the dynamical forecast and/or (2) strategies for acquiring new data for boundary condition updating during the forecast. The use of time-dependent objective analysis is essential. The 1983 forecast was a prototype experiment performed as part of the OPTOMA program (Ocean Prediction Through Observation, Modeling and Analysis) which plans a major forecasting experiment in the summer of 1984 when mixed-layer dynamics and satellite imagery will be added to the forecast scheme.

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1. Robinson, A. R. in *Eddies in Marine Science* (ed. Robinson, A. R.) 3–15 (Springer, Berlin, 1983).
2. Mooers, C. N. K., Piascek, S. A. & Robinson, A. R. (eds) *Proc. Ocean Prediction Workshop, Ocean Prediction: The Scientific Basis and the Navy's Needs*, Monterey (1981).
3. World Climate Research Program *Large-scale Oceanographic Experiments in the WCRP* Vol. 1, 92 (World Meteorological Organization, 1983).
4. Robinson, A. R. in *Large-Scale Oceanographic Experiments in the WCRP* Vol. 2, 357–388 (World Meteorological Organization, 1983).
5. Robinson, A. R. & Leslie, W. G. *Prog. Oceanogr.* (in the press).
6. Carter, E. F. *Rep. Met. Oceanogr.* No. 18 (Harvard University, Cambridge, 1983).
7. Miller, R. N., Robinson, A. R. & Haidvogel, D. B. *J. Comput. Phys.* 50, 38–70 (1983).
8. Mooers, C. N. K. & Robinson, A. R. *Science* 223, 51–53 (1984).

Light-induced reduction of natural iron(III) oxide and its relevance to phytoplankton

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Primary productivity in natural waters may sometimes be limited by the availability of iron^{1–4}. To understand the process it is necessary to know which form(s) of iron can be utilized by phytoplankton. Here we present evidence, from algal culture work and photochemical experiments, which suggests that the prevalent form of iron in most natural waters—particulate iron(III) oxide—is an adequate source of iron for algae, even though iron is taken up only in its soluble, ionic form⁵. This is because the oxide undergoes light-induced reduction, providing a continuous supply of soluble (available) iron(II). Humic substances (HS), co-precipitated with the oxide, participate in the reduction process by reducing the iron(III) directly and/or by maintaining it in a reducible state. These findings suggest that, at least in freshwaters, cessation of phytoplankton growth is rarely due to lack of iron.

The uptake of iron by phytoplankton, and the possibility of iron-limited growth, depend on the chemical speciation of the element^{6–7}. It has usually been assumed that algae take up soluble ionic species. However, the stable form of iron in oxic waters of neutral or near-neutral pH is Fe(III), which has a low solubility⁸. Therefore, algal growth might cease or be severely curtailed if, after initial growth had used up the small amount of soluble (available) iron, its replenishment was slow in relation to the further demands of the algae⁵. Whether such a situation actually arises depends on the lability of those iron species which, although unavailable *per se*, can be converted into available forms.

In algal culture work, iron is commonly added in a soluble chelated form, such as Fe(III)–EDTA⁹. Such chelates are thought to allow iron to be taken up efficiently through photodecomposition, whereby the chelated iron is reduced and released as soluble Fe(II)¹⁰. The latter can then be utilized by algae, either directly or after oxidation to ionic Fe(III) forms^{5,11}.

It has been suggested⁵ that the natural chelators for Fe(III) are HS. Although there is no firm evidence for soluble Fe(III) chelates in neutral or alkaline waters, iron oxides adsorb HS¹² and HS co-precipitate with natural iron oxide¹³. Furthermore, at acid pH values Fe(III) is readily reduced when HS are present, especially when exposed to light¹⁴. These facts prompted us to test naturally-occurring iron oxide for light-induced reduction and as an iron source for the common green alga *Monoraphidium contortum* (Thur. in Bréb.) Komárková-Legnerová. We believe this to be the first study of a well-characterized naturally-occurring form in relation to algal iron nutrition.

The natural iron oxide, ~10% by weight HS, accumulates in the hypolimnion of Esthwaite Water in Cumbria, UK, during

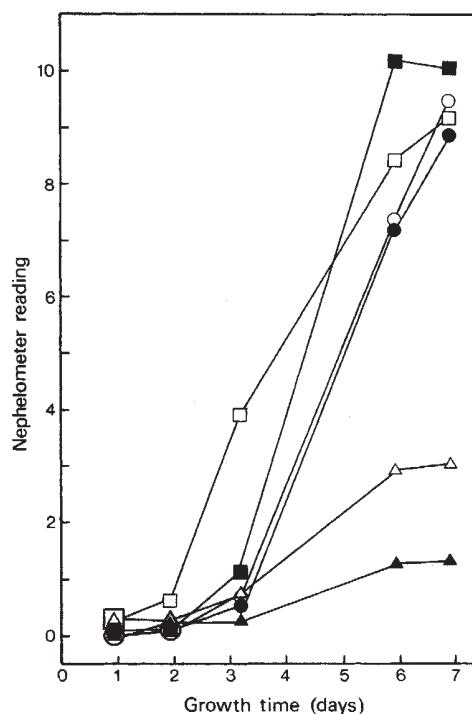


Fig. 1 Growth of *Monoraphidium* on various forms of Fe(III): Esthwaite Water iron oxide (○), synthetic Fe(III)–HS co-precipitate (●), Fe(III)–EDTA (□), fresh ferrihydrite (■), aged ferrihydrite (△), no added iron (▲). The algal growth medium consisted of NaCl, KNO₃, MgSO₄ and CaCl₂ (all 10^{−3} mol dm^{−3}), KH₂PO₄ (10^{−4} mol dm^{−3}) and Na₂CO₃ (7.5 × 10^{−4} mol dm^{−3}). When equilibrated with the atmosphere the pH was 8.1–8.2. The medium was filtered (0.22 µm Nuclepore) before the addition of iron (2 × 10^{−6} mol dm^{−3}). Aliquots (10 cm³) of medium were inoculated with a cultured strain of *Monoraphidium* (L188 from the Freshwater Biological Association Culture Collection; original isolate from Lough Neagh, 1969) at a concentration of ~2 × 10⁷ cells dm^{−3}. Before inoculation the *Monoraphidium* had been grown to the stationary phase in the same medium with 10^{−6} mol dm^{−3} fresh ferrihydrite as the iron source. The concentration of iron in the experimental tubes due to carry-over in the inoculum was ~10^{−8} mol dm^{−3}. The inoculated aliquots were contained in transparent polycarbonate tubes and were bubbled continuously with wet air to maintain constant pH and P_{CO2}. The tubes were incubated at 20 °C and illuminated with light (400–700 nm) at 216 µEinstein m^{−2} s^{−1} supplied from below by 4 × 40 W daylight fluorescent tubes. Algal growth was assessed by nephelometry, the scattered light being directly proportional to the concentration of cells in the range 0–3 × 10⁹ cells dm^{−3}; a nephelometer reading of 10 (arbitrary units) corresponds to 1.5 × 10⁹ cells dm^{−3}.

the summer, being formed through the oxidation of Fe(II) diffusing out of the sediment. The sample used in this work was collected from 1 m above the sediment of Esthwaite Water in July 1983, and was kept at neutral pH in the dark at 6 °C before use. Details of material sampled on earlier dates are given in ref. 13.

As well as the natural oxide, other forms of Fe(III), were tested. Aged ferrihydrite¹⁵ was prepared by neutralizing Fe(NO₃)₃ with NaOH and keeping the precipitate at pH 7 for 4 months at 6 °C in the dark. Fresh ferrihydrite was obtained by addition of a solution of Fe(NO₃)₃ directly to the culture medium in algal cultures or to the Tris–HCl buffer in the photochemical experiments (see below). A synthetic Fe(III)–humic co-precipitate (HS/Fe = 2, by weight) was prepared by oxidizing FeSO₄ in the presence of Penwhirn Reservoir HS¹⁶ and was stored in the same conditions as the aged ferrihydrite. Colloidal haematite (α-Fe₂O₃), goethite (α-FeOOH) and lepidocrocite (γ-FeOOH) were synthesized by methods given in refs 17, 18

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Table 1 Reaction of different forms of Fe(III) with bathophenanthroline disulphonate (BPDS)

Form of Fe(III)	% Fe reacted with BPDS after 16 h	
	Light	Dark
Esthwaite Water iron oxide	6	2
Fe(III)-HS co-precipitate	7	2
Fe(III)-EDTA	34	0
Ferrihydrite Fresh	39	11
Aged	2	1

Iron samples were incubated at a concentration of 2×10^{-4} mol dm $^{-3}$ in 5×10^{-4} mol dm $^{-3}$ BPDS and 0.02 mol dm $^{-3}$ Tris-HCl at pH 8.15 and 20 °C. When applied, illumination was as in the algal culture experiments (Fig. 1). The formation of Fe-BPDS complexes was monitored by spectrophotometry at 536 nm. No reduced iron was detectable at zero time.

and 19 respectively. The Fe(III) chelates of EDTA and the siderophore desferrioxamine B²⁰ were prepared with the chelates in twofold molar excess.

Figure 1 shows that, with a light regime which simulates natural conditions reasonably well, and at a total iron concentration (2×10^{-6} mol dm $^{-3}$) typical of natural waters, good growth of *Monoraphidium* was obtained with four of the forms of iron studied; Fe(III)-EDTA, the synthetic Fe(III)-HS co-precipitate, the fresh ferrihydrite and the natural Esthwaite Water iron oxide. The aged ferrihydrite was less effective but gave slightly better growth than was obtained with no added iron. Neither the crystalline oxides nor the Fe(III)-desferrioxamine B chelate improved growth.

Table 1 shows the results of photochemical experiments in which the different forms of iron were incubated, either illuminated or in the dark, at the same pH (8.15) as the algal cultures, in the presence of bathophenanthroline disulphonate (BPDS). BPDS strongly complexes Fe(II) and so would trap any Fe(II) produced before re-oxidation^{5,8,11}. The results for Fe(III)-EDTA are consistent with the well-established photodegradation of the chelate to give Fe(II) (ref. 10). The results for the other four types of iron which enhance algal growth are less clear-cut because in each case iron was to some extent trapped by BPDS in the dark. The dark-iron-BPDS reactions were probably due to the complexation by BPDS of the relatively labile Fe(III), followed by reduction to the BPDS-Fe(II) complex²¹ or, when HS were present, due to the reduction of the iron to Fe(II) which then reacted with BPDS. Notwithstanding the reactions in the dark, however, the enhanced production of BPDS-complexed iron when the samples were illuminated indicates that these Fe(III) species each underwent light-induced reduction to give Fe(II) which was subsequently trapped by BPDS. No Fe-BPDS complexation occurred with the crystalline oxides, nor with the Fe(III)-desferrioxamine B chelate.

The results in Table 1 and Fig. 1 show that those forms of Fe(III) which undergo light-induced reduction also support the growth of *Monoraphidium*, in accord with the hypothesis that algae utilize soluble, ionic iron. The Fe(III)-desferrioxamine B chelate is neither reduced nor does it support growth, showing that iron uptake does not depend merely on solubilization.

The results for the humic-free ferrihydrite samples show that HS are not essential for the production of Fe(II). However, the susceptibility of ferrihydrite to reduction decreases quite rapidly with time, as shown by the results for the fresh and aged samples in Table 1, and also by the observation that only 2 days' ageing at pH 8.15 was sufficient to decrease by 80% the amount of Fe trapped by BPDS in 16 h in illuminated conditions. In contrast, the synthetic Fe(III)-humic co-precipitate, in which the iron appears (by transmission electron microscopy) to be present largely as ferrihydrite, was both more readily reduced and better at supporting algal growth than the aged humic-free ferrihydrite, although the two samples were prepared at the same time and stored in the same conditions. The difference might be explained by the co-precipitated HS inhibiting the ageing of the ferrihy-

drite, or it may be that HS directly reduce aged ferrihydrite under illumination¹⁴.

Irrespective of their mechanism(s) of action, however, we conclude that co-precipitated HS can maintain the susceptibility of iron(III) oxide to light-induced reduction, and that this prolongs the availability to algae of Fe(III). The possible role of HS in making iron available was discussed by Shapiro⁶, but in terms of peptization of colloidal particles rather than reduction of Fe(III). The beneficial effects of HS on the growth in culture of a variety of algae²²⁻²⁵ may well have been due to the HS making iron more susceptible to reduction.

Wells *et al.*²⁶ considered that algal uptake of iron from synthetic iron(III) (hydr)oxides was related to the rate of (light-independent, non-reductive) dissolution of the solid. However, at neutral or alkaline pH, dissolution due to light-induced reduction is likely to be of much greater significance.

The mechanism for making iron available to algae demonstrated by our results is similar to one suggested²⁷ in relation to manganese uptake by marine phytoplankton, which was based on observations of light-induced reduction by marine HS of synthetic manganese dioxide.

In the epilimnion of Esthwaite Water, much of the particulate iron is present as amorphous iron(III) oxide particles similar in appearance and size to the hypolimnetic material studied above²⁸. Assuming that the epilimnetic iron is similarly susceptible to reduction, then at any one time during the summer months there is a large pool of iron (10^{-6} – 3×10^{-6} mol dm $^{-3}$) which can be made available to algae. The pool is continually replenished by iron oxide brought into the lake in inflows or diffusing upwards from the deep water or sediment. It is thus difficult to see how, in this lake, algal uptake could exhaust the supply of iron to the point where further growth was demonstrably iron-limited. Whether this applies to other natural waters remains to be seen. The effects on iron supply of total iron concentration, pH and light intensity require investigation, and other natural iron oxides need to be tested for light-induced reduction. Clearly, however, substantiation of suspected iron limitation in nature must take account of the reducible iron oxide pool in relation to the modest iron requirements of planktonic algae.

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- Gran, H. H. *Biol. Bull.* **64**, 159–181 (1933).
- Harvey, H. W. *J. mar. Biol. Ass.* **22**, 205–219 (1937).
- Goldberg, E. D. *Biol. Bull.* **102**, 243–248 (1952).
- Davies, A. G. *J. mar. Biol. Ass.* **50**, 65–86 (1970).
- Anderson, M. A. & Morel, F. M. M. *Limnol. Oceanogr.* **27**, 789–813 (1982).
- Shapiro, J. *J. Am. Wat. Wks. Ass.* **56**, 1062–1082 (1964).
- Shapiro, J. B. in *Chemical Environment in the Aquatic Habitat* (eds Golterman, H. L. & Clymo, R. S.) 219–228 (North-Holland, Amsterdam, 1967).
- Stumm, W. & Morgan, J. J. *Aquatic Chemistry* (Wiley, New York, 1970).
- Provasoli, L., McLaughlin, J. A. & Droop, M. R. *Arch. Mikrobiol.* **25**, 392–428 (1957).
- Hill-Cottingham, D. G. *Nature* **175**, 347–348 (1955).
- Owens, L. D. & Chaney, R. L. *Pl. Physiol.* **48**, 8 (1971).
- Tipping, E. *Geochim. cosmochim. Acta* **45**, 191–199 (1981).
- Tipping, E., Cooke, D. & Woof, C. *Geochim. cosmochim. Acta* **45**, 1411–1419 (1981).
- Miles, C. J. & Brezonik, P. L. *Environ. Sci. Technol.* **15**, 1090–1095 (1981).
- Schwertmann, U. & Taylor, R. M. in *Minerals in Soil Environments* (eds Dixon, J. B. & Weed, S. B.) 145–180 (Soil Science Society of America, 1977).
- Tipping, E. & Ohnstad, M. *Chem. Geol.* (in the press).
- Matijevic, E. & Scheiner, P. *J. Coll. Interface Sci.* **63**, 509–524 (1978).
- Atkinson, R. J., Posner, A. M. & Quirk, J. P. *J. phys. Chem.* **71**, 550–558 (1967).
- Sung, W. & Morgan, J. J. *Environ. Sci. Technol.* **14**, 561–568 (1980).
- Neilands, J. B. in *Inorganic Biochemistry* Vol. 1 (ed. Eichhorn, G. I.) 167–202 (Elsevier, Amsterdam, 1973).
- Lee, G. F. & Stumm, W. *J. Am. Wat. Wks. Ass.* **52**, 1567–1574 (1960).
- Prakash, A. & Rashid, M. A. *Limnol. Oceanogr.* **13**, 598–606 (1968).
- Prakash, A., Rashid, M. A., Jensen, A. & Subba Rao, D. V. *Limnol. Oceanogr.* **18**, 516–524 (1973).
- Giesy, J. P. *J. Phycol.* **12**, 172–179 (1976).
- Lange, W. *Proc. 13th Conf. Great Lakes Res.* **58**–70 (1970).
- Wells, M. L., Zorkin, N. G. & Lewis, A. G. *J. mar. Res.* **41**, 731–746 (1983).
- Sunda, W. G., Huntsman, S. A. & Harvey, G. R. *Nature* **301**, 234–236 (1983).
- Tipping, E., Woof, C. & Ohnstad, M. *Hydrobiologia* **92**, 383–393 (1982).

A new Lower Devonian plant and the early evolution of leaves

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The fossil record suggests that, by the end of the early Devonian, vascular plants had diversified considerably but they were still relatively simple in their organization¹⁻³. Most exhibit much-divided aerial shoots arranged in various patterns but lack clear differentiation into stems and leaves. I describe here a novel plant, discovered in the upper Lower Devonian of Gaspé, Quebec, Canada, whose anatomy is more complex and indicates a level of stem-leaf differentiation significantly more advanced than any known for trimerophytes or other taxa of this age. The presence of a lobed xylem, with mesarch protoxylem elements in major axes, and an elliptically shaped trace in first-order lateral axes in the new plant suggests that by the end of the early Devonian, the evolution of megaphyllous leaves has indeed begun, even though morphologically they were still much divided and non-laminate.

The anatomy of most vascular plants from the Lower Devonian consists of a solid terete or elliptical cylinder of tracheids with centrarch or exarch primary xylem maturation (except in lycophytes or lycophyte precursors with vascularized microphyllous leaves and lobed xylem). A variety of sporangial shapes and modes of attachment (terminal, lateral) occur and some possess unvascularized, usually multicellular emergences. No secondary tissues other than wound tissue are known and reproduction was homosporous or incipiently heterosporous. In contrast, middle and late Devonian plants show the first appearance of more complex stelar patterns, some morphological and/or anatomical distinction between stems and megaphyllous leaves, secondary tissues, arborescence, heterospory and the seed habit. By then, many major plant lineages had become evident, some of which can be linked, at least indirectly, to modern plant groups.

The new plant described here is represented by compression and permineralized specimens collected from the Battery Point Formation near Seal Rock Landing on the north shore of Gaspé Bay, Quebec, Canada. The age of the sediments is determined by dispersed spores which represent McGregor's *caperatus-emsianensis* dispersed spore assemblage zone⁴ of early Emsian age. On some compressions, short lengths of axes are permineralized by pyrite or limonite. Some axes also occur in isolated pyrite nodules. So far, only vegetative remains have been found in attachment, although associated fertile organs occur.

The new plant, although similar in external vegetative morphology and complexity to the contemporaneous (Lower Devonian) trimerophyte genera *Pertica*, *Trimerophyton* and, to some extent, *Psilophyton*⁵⁻⁷, differs significantly on close examination in details of branching pattern (Fig. 1a, b) as well as anatomy. The major axes, 0.7–1 cm wide, bifurcate into two equal-sized units (Fig. 1b, B). Each of these divides equally (isotomously) again or unequally (anisotomously) to produce distantly spaced, spirally arranged lateral branches (Fig. 1b, L). The latter divide anisotomously either just above their point of departure or more distally to produce small third-order axes. These divide one to three times and, where complete, terminate in recurved tips. All axes are covered with short (0.3–0.6 mm) hairs which are represented by punctiform scars on axis impressions.

The xylem consists of primary tissues forming a deeply three-lobed protostele (Fig. 1c). Three protoxylem areas, one near the end of each arm, are present, and a fourth is often located in the centre (Fig. 1c). This type of xylem was observed from sections of the major axes above and below isotomous bifurcations, at the points indicated in Fig. 1b.

Xylem maturation thus is mesarch; the protoxylem elements commonly are broken down to form lacunae. Protoxylem areas sometimes appear linked by a band of small cells which probably represent compressed metaxylem elements. The latter exhibit scalariform thickenings or oval and circular pits on all walls (Fig. 1d).

Lateral traces are helically arranged. Formation of a lateral trace involves: (1) tangential enlargement of the end of a xylem lobe, first to one side and then the other (Fig. 1f, g), (2) enlargement and division in protoxylem tissue (Fig. 1g, h) and (3) separation of outer protoxylem and associated metaxylem elements (Fig. 1h, e). The tangentially elongate lateral trace usually remains elliptical in shape, with centrally located protoxylem, after leaving the cortex. These traces supply the lateral branches resulting from anisotomies (branches labelled L in Fig. 1b).

No early Devonian plant exhibits this particular combination of a lobed vascular strand, mesarch primary xylem maturation and type of lateral trace production. Several groups of middle Devonian plants, namely the Aneurophytales (Progymnospermopsida), Iridopteridales (sensu Stein, 1982) and Cladoxylales⁸⁻¹³ have variously lobed xylem with protoxylem lacunae surrounded either by remnants of protoxylem or by metaxylem. In these plants, vascular traces to lateral branches usually depart from the lobe tips; at some level the lateral traces differ from the parent axis in size or shape and where different are believed to indicate the presence of leaves. These plants can be distinguished from each other and from the new plant in number and location of protoxylem areas, shape and mode of production of lateral traces, and in the presence (in aneurophytes) of secondary tissues. The new plant might be regarded as a trimerophyte derivative leading towards certain of these younger plant groups, such as the Aneurophytales or Iridopteridales. Other plant groups such as the Cladoxylales and/or ferns probably evolved independently from a trimerophyte ancestor, perhaps at different times.

The anatomy of the new plant offers evidence concerning differentiation of stems leading to the establishment of leaves. In most early Devonian plant groups, evolution of megaphyllous leaves is regarded as being at a very early stage, manifested mainly as variability in branching patterns of stem-like axes. Plants with undoubtedly laminate, megaphyllous leaves occur in the Upper Devonian^{14,15}, although rare occurrences of incompletely preserved laminate, apparently megaphyllous leaves (*Platylphyllum*) of uncertain affinities have been reported from the Emsian^{1,14}. It has been postulated^{16,17} that the trimerophytes exhibit early transitions of branch systems to megaphyllous leaves, as follows: (1) in *Psilophyton*, variation in dichotomous to pseudomonopodial branching patterns; (2) in *Pertica* and *Trimerophyton*, the development of a distinct, robust major axis with subordinate lateral branches; and (3) in the latter two genera a tendency for regularly arranged, but pseudomonopodially divided lateral branches. Where anatomy is known, no major change in vascular strand shape occurs in the various orders of branching, except for a slight difference in shape in strands departing to fertile branches in *Psilophyton dawsonii*¹⁶. The new plant demonstrates a pronounced anatomical change from the lobed xylem of the main axis to an elliptically shaped, first-order lateral branch trace. Comparable changes in vascular strand shape in younger fossil plants, or extant ones, occur between stem and leaf.

The transition from an elliptical centrarch xylem strand, typical of trimerophytes, to a lobed, mesarch one as is present in the new plant and several Middle–Upper Devonian ones of various affinities is here considered possible by loss during development of centrally located protoxylem, leaving separate peripheral strands, and by variable metaxylem development. An intermediate condition may occur in *Psilophyton dawsonii* in those axes from which numerous vegetative branches depart in quick succession and which exhibit a somewhat lobed protoxylem region and irregularly shaped metaxylem. Additionally, a lobed xylem with protoxylem areas in the lobes, as found in the

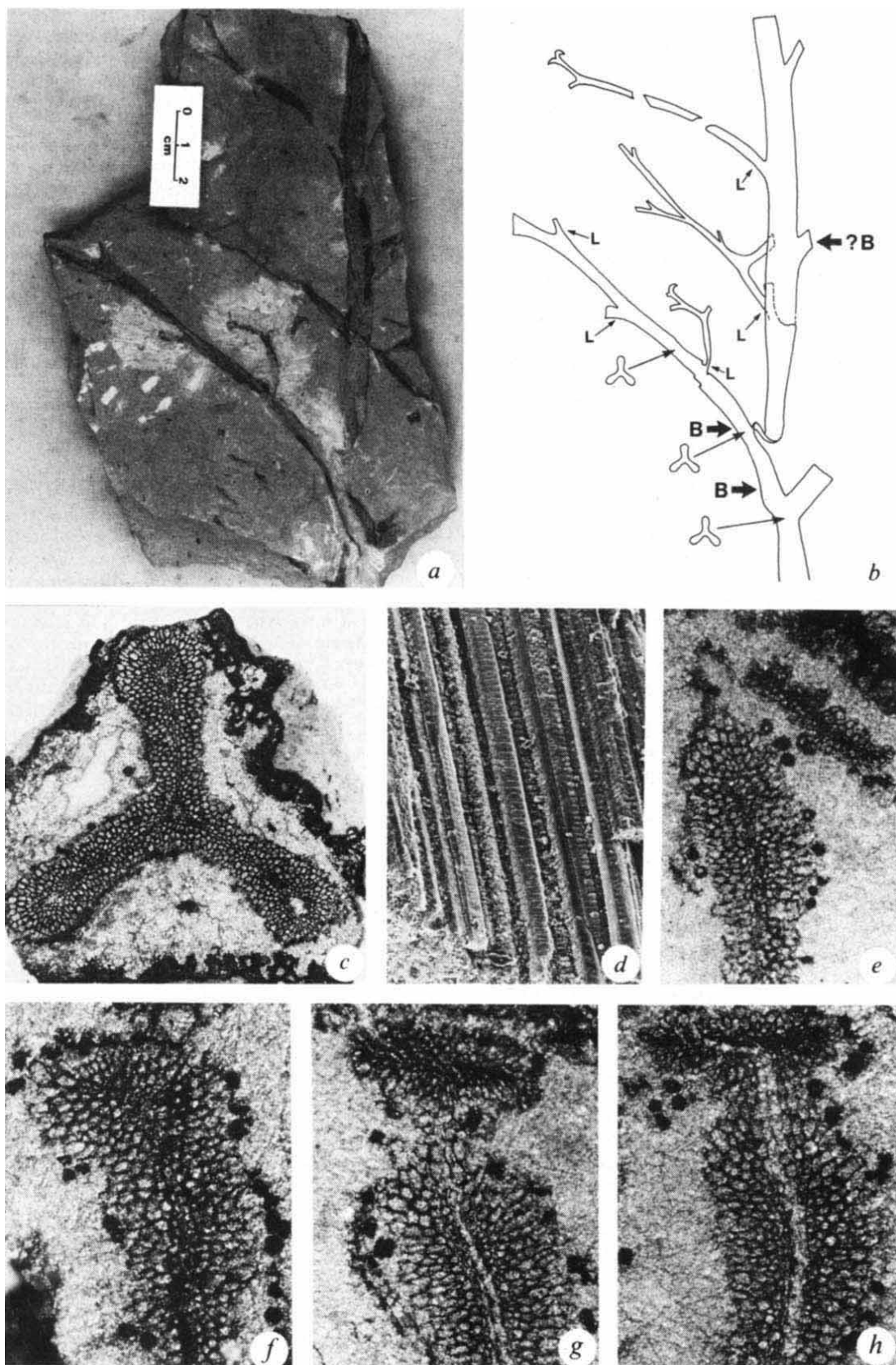


Fig. 1 *a*, Compression/impression showing major components of branching: note two equal bifurcations of the main axis near the base, and production of lateral branches distally. *b*, Line drawing based on tracing photographs of part and counterpart of specimen in *a*. The lateral branch in the middle of the drawing was exposed by splitting of the upper portion of the specimen in *a*. B, Bifurcation of major axis; L, lateral branch; three-lobed symbols indicate regions where permineralized axes were removed. *c*, Transverse section of major axis obtained from specimen in *a*, showing deeply three-lobed stele, protoxylem lacunae and a band of narrow metaxylem cells in the centre. $\times 20$. *d*, Scanning electron micrograph of pyritized tracheids in longitudinal view, showing tapered end walls (lower right) and scalariform to oval pits. *e*, One lobe of a major axis and an elliptical lateral trace: the trace was produced by the sequence in *f-h*. $\times 42$. *f-h*, Stages in the production of lateral trace. *f*, Xylem at tip of lobe expands to one side. $\times 56$. *g*, Xylem is expanded in both directions; trace is beginning to become detached from lobe. $\times 55$. *h*, Trace further detached from lobe and nearly fully formed; this becomes separate as shown in *e*. $\times 60$.

new plant, may be linked to the very regular pattern of lateral branch departure. In view of this evidence, it is interesting to consider once again what type of xylem existed in plants like the trimerophytes *Pertica* and *Trimerophyton*, which also show a regular pattern of lateral branch departure.

In summary, the new plant, while resembling known Lower Devonian plants in general morphology, is more complex anatomically but can be logically derived from them. Further, it suggests that appendicular structures or leaves were already being differentiated at that time, even though morphologically they still were much divided and non-laminate.

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1. Chaloner, W. G. & Sheerin, A. *Spec. Pap. Palaeontol.* 23, 145–161 (1979).
2. Banks, H. P. *Can. J. Bot.* 59, 1292–1296 (1981).
3. Banks, H. P. in *Biostratigraphy of Fossil Plants* (eds Dilcher, D. & Taylor, T. N.) 1–24 (Dowden, Hutchinson & Ross, Stroudsburg, 1980).
4. McGregor, D. C. *Palaeontographica* 142B, 1–77 (1973).
5. Kasper, A. E. & Andrews, H. N. *Am. J. Bot.* 59, 897–911 (1972).
6. Granoff, J. A. *et al. Palaeontographica* 155B, 119–128 (1976).
7. Gensel, P. G. *Palaeontographica* 168B, 81–99 (1979).
8. Beck, C. B. *Rev. Palaeobot. Palynol.* 21, 5–23 (1976).
9. Stein, W. *Bot. Gaz.* 143, 401–416 (1982).
10. Matten, L. & Banks, H. P. *Am. J. Bot.* 53, 1020–1028 (1966).
11. Scheckler, S. E. & Banks, H. P. *Am. J. Bot.* 58, 737–751 (1971).
12. Matten, L. C. *Am. J. Bot.* 60, 619–630 (1973).
13. Stein, W. *Palaeontology* 25, 605–622 (1982).
14. Chaloner, W. G. *Biol. Rev.* 45, 353–377 (1970).
15. Galtier, J. *Palaeontographica* 180B, 1–38 (1981).
16. Banks, H. P. *et al. Palaeontogr. Am.* 8, 75–127 (1975).
17. Gensel, P. G. *Brittonia* 29, 14–29 (1977).

Recurrent patterns of natural selection in a population of Darwin's finches

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The adaptive significance of morphological traits can be assessed by measuring and identifying the forces of selection acting on them. Boag and Grant¹ documented directional selection in a small population of Darwin's medium ground finches, *Geospiza fortis*, on I. Daphne Major, Galápagos, in 1977. Large beak and body size were favoured at a time of diminishing food supply and high adult mortality. We show here that in two subsequent periods of moderate to high adult mortality (1980 and 1982), the population was subject to the same selection. We have used a recently developed technique² to ascertain the targets of direct selection. Beak depth and body weight were commonly under direct selection to increase but, surprisingly, beak width was directly selected to decrease, over all three periods of mortality. The results have implications for our understanding of evolutionary change in morphological traits of Darwin's finches^{3–6}.

We consider three 18-month periods of adult disappearance, mainly due to mortality^{1,3}, between June 1976 and December 1982 (Table 1). Each period was associated with a year of low rainfall (Table 1). Less than 5% of adult mortality occurred in the remaining 24 months (January 1978–June 1979 and January–June 1981). Only adults (at least 1 year old) which had been ringed and measured before the periods of high mortality are included in the analyses. Survival of ringed and measured birds was assessed by a detailed census at the end of each period^{1,3}. Seed abundance was estimated at this time by counting seeds on plants and in the soil in 50 randomly placed quadrats^{1,3}. Four characters (body weight in grams, and beak length, beak depth and beak width in millimetres) have been measured by different observers in a consistent fashion^{3,4}. The characters are

highly and significantly positively correlated^{4–6}. Before each analysis, data were log_e-transformed and then standardized to have zero mean and unit variance.

Table 2 shows the vectors of directional selection differentials (*s*) and the directional selection gradients (β)² associated with mortality in each of the three periods. A selection differential is the difference between the mean value of the character before and after selection, here measured in standard deviation units. The selection gradient comprises the partial regression coefficients of relative fitness (survival) on each character. Relative fitnesses are obtained by dividing absolute fitnesses (0 or 1) by the mean absolute fitness², that is, the proportion surviving. Thus, each partial regression coefficient measures intensity of selection acting directly on each character, apart from phenotypic responses due to selection on other correlated and measured characters². After 1978 the ages of birds were known, so we repeated the analysis separately for each age cohort to test for age-specific effects (Table 3).

For all characters and periods, selection differentials are significantly positive, or they are not significantly different from zero: survivors are often larger than those individuals that disappear (Tables 2, 3). The most intense selection in this direction was in 1976–77, when the lowest number of individuals (15%) survived (Table 2). The selection gradients indicate that increases in all measured characters are due almost entirely to selection on beak depth and/or body weight (or to selection on an unmeasured character with which these are highly correlated). Beak length is usually not a target of direct selection, but it increases as a correlated response to selection on beak depth and/or body weight. The partial regressions of relative fitness on beak width are usually negative and often significant: this result was not expected in view of the net increases in the sizes of all traits in 1976–77. Thus, beak shape, as well as size, is subject to selection.

In each of the three periods of mortality, partial regression coefficients of relative fitness on beak depth and body weight are positive and partial regression coefficients of relative fitness on beak width are negative (Tables 2, 3). When the selection over the 1977 drought is divided into three consecutive 6-month periods, this pattern is observed within each period (Table 4). It is also observed when selection on each sex is analysed separately⁵.

The repeatedly observed direct selection favouring a small beak width was often intense. It was sufficient to counteract strong selection favouring large beak depth and/or body weight in the 1978 cohort in both 1979–80 and 1981–82, resulting in small selection differentials for all characters, but not in the pre-1976 cohort in 1981–82 (Table 3).

Selection events can be interpreted in terms of feeding conditions. Seed supplies decline during a drought¹, but hard seeds are proportionally common (Table 1). Only the larger individuals can crack these seeds^{1,7,8}. Among these large individuals, efficiency at cracking the woody *Tribulus* fruits is a positive function of beak size⁷. Selection for large beak and body size associated with starvation has previously been related to the superior seed handling ability of large individuals¹. The demonstration here that beak depth is a direct target of selection strengthens this interpretation.

A plausible hypothesis for the selection to decrease beak width is that a relatively narrow beak is favoured in the specialized feeding on *Tribulus* fruits: individuals crack the fruits by placing the side of the beak across a corner and twisting⁷. *Tribulus* is an important food for *G. fortis* during droughts because it remains common (Table 1) and, unlike *Opuntia*, is not defended or consumed by the cactus ground finch, *Geospiza scandens*, the only other common finch on the island. *Tribulus cistoides* may have been introduced to the Galápagos in the past 450 yr^{7,9}. If so, the directional selection pressures on beak width that we have detected are relatively new.

This study has shown that selection repeatedly favours individuals with large beak depth and/or body weight in recurring drought conditions. It has also uncovered an unsuspected

Table 1 Selection periods, population sizes before and after each selection period, and seed biomass at the end of drought years (g per 50 m²)

Drought year	Period studied	Rainfall (mm)	Population sizes		Small seeds	Seed abundance	
			Before	After		<i>Opuntia echios</i>	<i>Tribulus cistoides</i>
1977	June 1976 to December 1977	24.0	1,200	200	1.2	13.6	117.9
1980	June 1979 to December 1980	53.5	260	210	1.7	184.2	130.6
1982	June 1981 to December 1982	51.1	200	150	0.5	21.0	38.6

Seeds are divided into miscellaneous small seeds, and a medium-size seed (*Opuntia*) and a large seed (*Tribulus*), both of which require significant force to crack and cannot be handled by all the finches^{1,7,8}. All rain fell in January–May of each period.

Table 2 Standardized selection differentials (*s*) and standardized selection gradients (β) for periods of mortality

	1976–77		1979–80		1981–82	
	<i>s</i>	$\beta \pm \text{s.e.}$	<i>s</i>	$\beta \pm \text{s.e.}$	<i>s</i>	$\beta \pm \text{s.e.}$
Weight (g)	<u>0.62</u>	<u>0.51</u> $\pm$ 0.14	0.05	0.08 $\pm$ 0.06	<u>0.15</u>	0.13 $\pm$ 0.08
Beak length (mm)	<u>0.49</u>	0.17 $\pm$ 0.18	0.02	–0.04 $\pm$ 0.07	<u>0.13</u>	0.06 $\pm$ 0.09
Beak depth (mm)	<u>0.60</u>	<u>0.79</u> $\pm$ 0.23	0.03	0.13 $\pm$ 0.10	<u>0.12</u>	0.17 $\pm$ 0.12
Beak width (mm)	<u>0.49</u>	–0.47 $\pm$ 0.21	0.01	–0.14 $\pm$ 0.09	0.08	–0.20 $\pm$ 0.12
<i>R</i> ²		<u>0.09</u>		0.02		<u>0.06</u>
Sample size		640		192		197
Proportion surviving		0.15		0.78		0.65

All values lie between –1 and 1. Sample sizes refer to birds alive at the beginning of each period. Significance of selection differentials was assessed by *t*-tests comparing survivors with those who disappeared. Significant *R*² values, partial regression coefficients and selection differentials are underlined (*P* < 0.05).

Table 3 Standardized selection differentials (*s*) and standardized selection gradients (β) for two cohorts over the 1980 and 1982 drought periods

	1979–80				1981–82			
	Pre-1976		1978		Pre-1976		1978, 1979	
	<i>s</i>	$\beta \pm \text{s.e.}$	<i>s</i>	$\beta \pm \text{s.e.}$	<i>s</i>	$\beta \pm \text{s.e.}$	<i>s</i>	$\beta \pm \text{s.e.}$
Weight (g)	0.03	0.05 $\pm$ 0.08	0.11	0.06 $\pm$ 0.08	<u>0.24</u>	0.07 $\pm$ 0.12	0.08	0.15 $\pm$ 0.09
Beak length (mm)	–0.10	–0.20 $\pm$ 0.09	0.11	0.08 $\pm$ 0.09	<u>0.28</u>	0.16 $\pm$ 0.14	0.02	0.01 $\pm$ 0.11
Beak depth (mm)	–0.05	0.03 $\pm$ 0.12	<u>0.12</u>	<u>0.37</u> $\pm$ 0.15	<u>0.27</u>	0.16 $\pm$ 0.19	0.01	0.16 $\pm$ 0.15
Beak width (mm)	–0.04	0.05 $\pm$ 0.11	0.05	–0.39 $\pm$ 0.15	<u>0.24</u>	–0.09 $\pm$ 0.19	–0.04	–0.29 $\pm$ 0.15
<i>R</i> ²		0.08		<u>0.11</u>		<u>0.15</u>		0.05
Sample size		92		100		85		112
Proportion surviving		0.84		0.73		0.64		0.67

'Pre-1976' includes all birds hatched in 1975 or earlier. For the 1982 drought period the 17 birds hatched in 1979 are included with the 1978 cohort. Significant values (*P* < 0.05) are underlined.

Table 4 Standardized selection differentials (*s*) and standardized selection gradients (β) over the drought of 1977, divided into three consecutive periods

	June–December 1976		December 1976 to June 1977		June–December 1977	
	<i>s</i>	$\beta \pm \text{s.e.}$	<i>s</i>	$\beta \pm \text{s.e.}$	<i>s</i>	$\beta \pm \text{s.e.}$
Weight (g)	<u>0.05</u>	0.03 $\pm$ 0.04	<u>0.28</u>	<u>0.23</u> $\pm$ 0.08	<u>0.28</u>	<u>0.29</u> $\pm$ 0.12
Beak length (mm)	<u>0.06</u>	–0.01 $\pm$ 0.05	<u>0.21</u>	–0.17 $\pm$ 0.10	<u>0.22</u>	–0.04 $\pm$ 0.14
Beak depth (mm)	<u>0.08</u>	<u>0.19</u> $\pm$ 0.07	<u>0.30</u>	<u>0.43</u> $\pm$ 0.13	<u>0.23</u>	0.19 $\pm$ 0.19
Beak width (mm)	0.04	–0.15 $\pm$ 0.06	<u>0.24</u>	–0.19 $\pm$ 0.12	<u>0.21</u>	–0.14 $\pm$ 0.17
<i>R</i> ²		<u>0.02</u>		<u>0.09</u>		<u>0.09</u>
Sample size		640		442		194
Proportion surviving		0.69		0.44		0.50

The periods were defined by the times at which a census of surviving finches was taken. The data were not re-standardized from the first selection period. Significant values (*P* < 0.05) are underlined.

opposing selection in favour of small beak width. Given the high heritabilities of these characters^{4,10}, there is a strong potential for microevolutionary change between generations. The potential is realized⁴, but only to a small degree for two reasons. First, there is a high positive genetic correlation, estimated to be between 0.9 and 1.0 (refs 4, 6), between the two beak traits that are selected in opposite directions. Second, net selection

on beak depth and body weight over a whole life cycle is probably small because of opposing selection pressures at other stages of the life cycle, most notably in association with juvenile mortality: small individuals have a selective advantage over large individuals in their first 2–10 months, apparently for metabolic reasons³.

Results of the study support an adaptive interpretation given

to beak size and shape in this population of Darwin's finches^{6,11-13}.

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1. Boag, P. T. & Grant, P. R. *Science* **214**, 82-85 (1981).
2. Lande, R. & Arnold, S. J. *Evolution* **37**, 1210-1226 (1983).
3. Price, T. D. & Grant, P. R. *Evolution* **38**, 483-494 (1984).
4. Boag, P. T. *Evolution* **37**, 877-894 (1983).
5. Price, T. D. *Am. Nat.* **123**, 500-518 (1984).
6. Price, T. D., Grant, P. R. & Boag, P. T. in *Population Biology and Evolution* (eds Wöhrmann, K. & Löschcke, V.) (Springer, Berlin, 1984).
7. Grant, P. R. *Anim. Behav.* **29**, 785-793 (1981).
8. Grant, P. R., Grant, B. R., Smith, J. N. M., Abbot, I. J. & Abbott, L. K. *Proc. natn. Acad. Sci. U.S.A.* **73**, 257-261 (1976).
9. Porter, D. M. *Rhodora* **69**, 455-456 (1967).
10. Boag, P. T. & Grant, P. R. *Nature* **274**, 793-794 (1978).
11. Lack, D. *Darwin's Finches* (Cambridge University Press, 1983).
12. Grant, P. R. *Am. Scient.* **69**, 653-663 (1981).
13. Boag, P. T. & Grant, P. R. *Biol. J. Linn. Soc.* **21** (in the press).

Functions of the canal system in the rotaliid foraminifer, *Heterostegina depressa*

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Advanced fossil and living rotaliacean foraminifera are characterized by canal systems located within the walls of their plurilocular calcareous tests. Although these canals were described in detail during the nineteenth century¹⁻⁴, their biological functions have remained obscure. A study of the Recent nummulitid, *Heterostegina depressa*, has now illuminated the role of the canal system and allowed us to interpret similar structures seen in related foraminifera of Tertiary age. It proves to be a structure of fundamental importance for locomotion, growth, excretion, reproduction and protection.

Although foraminiferal tests are characterized by a multiplicity of shapes, wall structures and construction materials, in only a few taxa do we understand their functional roles well enough to demonstrate relationships to feeding methods or environments (substrates)^{5,6}. The only group in which structural features can be explained as convergent adaptations to special modes of life are the larger symbiont-bearing benthic foraminifera (that is, members of the Miliolacea and Rotaliina). The transparency of the test wall and the high ratio of surface area to volume in those with flattened tests, provide the morphological basis for a highly advantageous algal-foraminiferal symbiosis⁷⁻⁹. We have examined the canal system within the symbiont-bearing Recent nummulitid, *H. depressa*, in which the test walls replace the primary aperture seen in other foraminifera.

H. depressa d'Orbigny inhabits the warm, shallow, nutrient-poor seas of the tropics and subtropics, where the light flux is relatively uniform throughout the year. This species exhibits marked physiological and morphological trimorphism: gamonts are up to 4 mm in diameter, while agamonts are up to 20 mm and schizonts up to 3 mm across. Although laboratory cultures¹⁰ in which all three forms reproduced were a prerequisite for this investigation, specimens from the sea around Hawaii were also used.

In most multilocular foraminifera, successive chambers are connected by a single foramen, the last-formed chamber opening

into the surrounding medium through the terminal foramen (aperture). Many species of rotaliid foraminifera have canal systems of different kinds within the chamber walls, and in genera such as *Operculina* these canals allow communication between the undivided chamber cavities and the lateral surfaces of the test. The subdivided chamber cavities (chamberlets) of *Heterostegina* communicate with the lateral surfaces in a similar manner. Nummulitids also have a three-dimensional network of canals (continuous with the rest of the canal system) within the thickened peripheral keel (marginal cord), and this opens into the ambient seawater.

It is known that nummulitids do not usually have true primary or secondary apertures¹¹, and few, if any, type descriptions of *Heterostegina* species establish their presence. This is not surprising, since the canaliculate marginal cord together with the canals of the chamber and chamberlet walls serve as an effective substitute. We have, however, observed apertures of various shapes and sizes in at least some individuals (see ref. 12).

The morphology of the canal system has recently been investigated by optical and scanning electron microscopy, and its genesis explained; it has also been used taxonomically^{9,11,13,14}. The canal system permits the extrusion of pseudopodia from any point of the marginal cord, even when protoplasm has been retracted from the peripheral chambers. We regard this as its primary function, since it provides the animal with a physiological radial symmetry. In foraminifera lacking a canaliculate marginal cord, pseudopodia emerge only from a single point in the test (the aperture), and then only if the protoplasm has not been retracted from the last chamber. As the extrusion of pseudopodia is the primary function of the aperture in other groups of foraminifera, it seems likely that its secondary functions are also taken over by the canal system in the Nummulitidae. That this is so is demonstrated here for the first time.

In *H. depressa*, the terminal openings of the canals in the marginal cord function as a multitude of small primary apertures and extrude protoplasm which forms the template for the new chamber (Fig. 1). Although this process has been described previously¹², only since it was recorded on film¹⁵ has the significance of the canal system been appreciated. Nummulitids such as *H. depressa*, *Heterocyclus tuberculata* Hottinger and *Operculina ammonoides* (Schröter) would probably be unable to construct large chambers with only a single exit for protoplasm.

H. depressa is nourished by photosynthates obtained from symbiotic algae contained within the cell, and by the continuous digestion of a proportion of the total population of symbionts¹⁶. Digestion occurs throughout the test, and waste products are transported by the protoplasm from older to younger chambers. Eventually, they are excreted through the openings in the marginal cord on the terminal chamber (Fig. 2) and through the aperture where this exists. The canal system thus provides for the removal of waste matter.

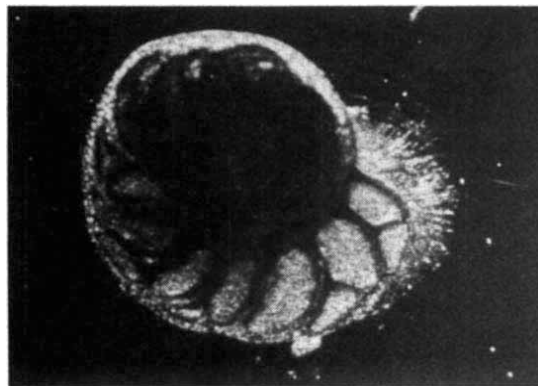


Fig. 1 Process of chamber formation in *H. depressa*. Protoplasmic filaments in large numbers emerge from the canals of the marginal cord of the youngest chamber in order to construct the template of a new chamber. Size of specimen 900 μm .

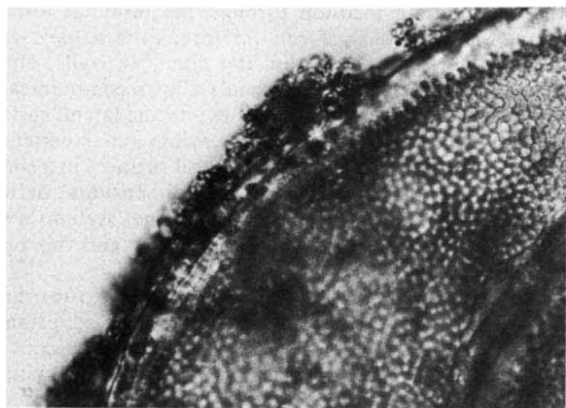


Fig. 2 Part of the lateral surface of the youngest chamber of *H. depressa* with accumulations of excrement on the marginal cord of the test. The 1–2 μm -sized grains have been excreted through the canals of the marginal cord.

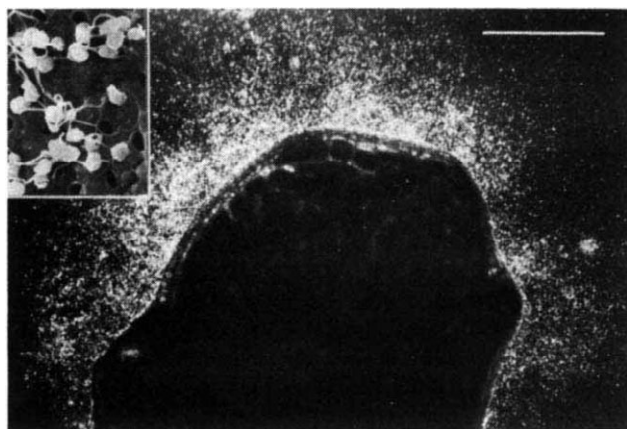


Fig. 3 Gamont of *H. depressa* from 100 m water depth off Honolulu, Hawaii, releasing gametes (scale bar, 420 μm). Inset: The scanning electron micrograph of the test surface shows pores and biflagellate gametes which were released through the canals. Size of the gametes 1 μm owing to shrinkage during fixation; size of living gametes 2.5 μm .

The canal system also functions during sexual reproduction. The 2.5- μm gametes are released mainly through the canal system of the marginal cord (Fig. 3). During asexual multiple fission of the schizont and agamont, the protoplasm, along with the symbionts, evacuates the test and subsequently divides into hundreds or thousands of daughter cells in the ambient seawater. Microcinematographic time-lapse photographs¹⁷ have shown that the protoplasm emerges from the lateral surfaces of the parent test via the canal system (Fig. 4a–c). The symbiotic diatoms undergo severe deformation as they are transported through the 2–3- μm wide sutural canals. This is possible because they do not possess silica frustules but only membranous cell envelopes; normally, they are 8–11 μm long, 4–5 μm wide and very variable in shape¹⁸; they are thus well-adapted to this mode of transport. The outflow of the entire protoplasm through the long and narrow canal system takes a comparatively short time (38 min–4 h 16 min).

Throughout most of its life, *H. depressa* is covered by a transparent sheath attached to the substrate by elastic processes. This prevents transport in turbulent water and also protects the chambers during the growth phases which occur internally. Immature individuals leave their sheaths at intervals and replace them with larger ones^{12,19,20}. Light microscope observations show that the secretion of the sheath lasts only a few hours, the material being passed through openings of the canal system onto the surface of the test. The canal system as a whole may therefore be said to provide the morphological basis for the rapid formation of this organic protective structure.

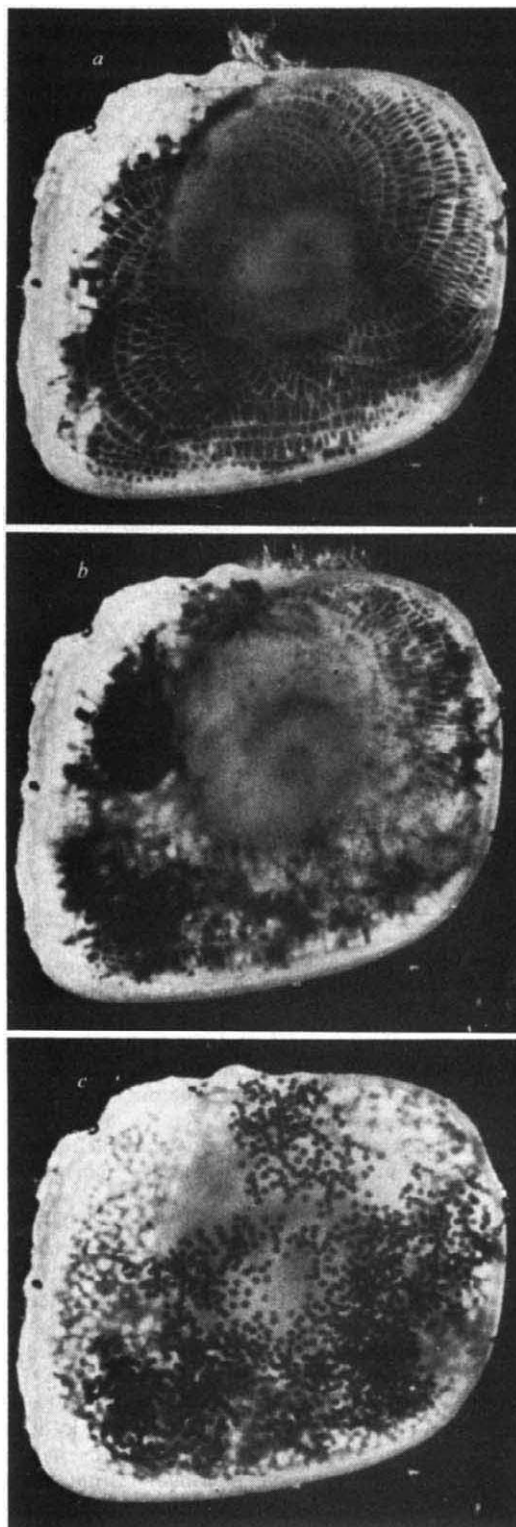


Fig. 4 Microcinematographic shots¹⁷ of an agamont of *H. depressa* from 45 m water depth, Island of Maui, Hawaii (size 9.8 mm). The protoplasm emerges from the test and then divides into the daughter cells. a, The protoplasm has retracted from the peripheral part of the calcareous test, thus indicating the beginning of multiple fission. Its dark coloration, caused by the symbiotic diatoms, contrasts with the colourless walls of the chambers and chamberlets. b, The protoplasm has evacuated most of the test through the canal system, and now covers both lateral surfaces. c, The protoplasm has divided into the daughter cells. Juveniles lying below the maternal test are also visible. Duration of evacuation process 2 h 17 min.

The canal system thus proves to be important for all the principal biological functions of the protoplasm.

Although the flattened and translucent nummulitid test is well adapted to its function as part of a symbiotic association, it also encloses a large volume of protoplasm (plus symbionts) that has to communicate with the outside world. This connection is effected by the canal system which provides physiological continuity between the internal protoplasm and the surrounding seawater.

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1. Carpenter, W. B. *Q. Jl. geol. Soc. Lond.* **6**, 21–39 (1850).
2. Carpenter, W. B. *Phil. Trans. R. Soc.* **149**, 1–41 (1859).
3. Carter, H. J. *Ann. Mag. nat. Hist. Ser. 2*, Vol. 10, 161–176 (1852).
4. Carpenter, W. B., Parker, W. K. & Jones, T. R. *Introduction to the Study of the Foraminifera*, 1–319 (Hardwicke, London, 1862).
5. Haynes, J. R. *Foraminifera* 1–433 (Macmillan, London, 1981).
6. Lipps, J. H. in *Biotic Interactions in Recent and Fossil Benthic Communities* (eds Tevesz, M. J. S. & McCall, P. L.) 331–376 (Plenum, New York, 1983).
7. Haynes, J. R. *Contr. Cushman Fdn Forum. Res.* **16**, 40–43 (1965).
8. Ross, C. A. *Proc. 2nd int. Coral Reef Symp.* **1**, 327–333 (Great Barrier Reef Committee, Brisbane, 1974).
9. Hottinger, L. in *Foraminifera* Vol. 3 (eds Hedley, R. H. & Adams, C. G.) 203–266 (Academic, London, 1978).
10. Röttger, R. *Mar. Biol.* **15**, 150–159 (1972).
11. Hottinger, L. *Mem. Mus. Natn. Hist. nat. Paris nouv. sér. C*, **40**, 3–159 (1977).
12. Spindler, M. & Röttger, R. *Mar. Biol.* **18**, 146–159 (1973).
13. Spindler, M. *J. Foram. Res.* **8**, 319–333 (1978).
14. Billman, H., Hottinger, L. & Oesterle, H. *Schweiz. Palaeont. Abh.* **101**, 71–113; 1–39 (1980).
15. Röttger, R. & Inst. Wiss. Film, Film C 1451 des IWF, Göttingen (1982); *Publ. Wiss. Film., Sekt. Biol.*, Ser. 15, No. 21, 1–15 (1982).
16. Schmaljohann, R. thesis, Univ. Kiel (1980).
17. Röttger, R. & Inst. Wiss. Film, Film C 1506 des IWF, Göttingen (1983); *Publ. Wiss. Film., Sekt. Biol.* (in the press).
18. Schmaljohann, R. & Röttger, R. *J. mar. Biol. Ass. U.K.* **58**, 227–237 (1978).
19. Röttger, R. *Mar. Biol.* **21**, 127–138 (1973).
20. Röttger, R. & Richwien, M. *Abstr. 5th int. Congr. Protozool.*, New York (1977).

Peripheral injury enhances central regeneration of primary sensory neurones

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The success of peripheral and fetal neural tissue in promoting outgrowth of axons from the adult mammalian central nervous system^{1–4} has tended to focus attention on local interactions between extending axons and their environment. The contribution to axon regeneration of biochemical and morphological changes in injured neurones^{5–7} is more difficult to evaluate. We report here that long spinal axons of primary sensory neurones are 100 times more likely to regenerate into peripheral nerve grafts if their peripheral axons are also cut. Regenerative behaviour at the axon tip seems to be strongly influenced by inducible events in the nerve cell.

In each of 11 rats, 6–8 weeks old, the dorsal columns were incised bilaterally after high cervical laminectomy and a segment from the right sciatic nerve was transplanted in the midline to the injured spinal cord. Such autogenous grafts provide a favourable milieu for spinal axons that usually fail to regenerate in their natural neuroglial environment^{2,8,9}. Two to three months after the operation, retrograde tracing with horseradish peroxidase (HRP) was used to identify neurones with axons regenerating from the spinal cord (Fig. 1). Particular attention was paid to the L4 and L5 dorsal root ganglia (DRG), which contain many large neurones projecting centrally to the brain stem and peripherally to the sciatic nerve. Control experiments with crushing of grafts near the spinal cord at the time of injection of HRP

excluded the possibility of false-positive labelling by diffusion or vascular spread. It was assumed that a neurone was labelled only if its spinal axon had entered a peripheral nerve graft and grown several millimetres or more within it. On the left, the L4 and L5 DRG usually contained no labelled neurones, and the most per pair was 10. On the right, all L4 or L5 DRG contained labelled neurones and the maximum for a pair of ganglia was 682. In all 22 matches between L4 or L5 ganglia the count was always higher on the right than on the left. The mean count per ganglion on the right (114) was more than 100-fold greater than on the left (<1). To provide a rough estimate of the size of the pool of lumbar sensory neurones with axons projecting to the vicinity of the dorsal column nuclei, a mixture of wheat germ agglutinin and HRP was injected into the dorsal columns at high cervical level in normal rats. The highest number of labelled neurones counted in a single L5 DRG was 840. In DRG of some grafted animals, the number of neurones with regenerating spinal axons exceeded 50% of this count.

Similar grafting and labelling techniques were used on four other groups of rats (Fig. 2a) receiving different combinations of treatments to the two sciatic nerves. When neither sciatic nerve was cut (in Lewis rats with isogenous grafts), almost no regeneration was detected from lumbar DRG; when both sciatic nerves were cut, the mean count for labelled neurones in L4 + L5 DRG on each side was 385 and the difference between counts on the two sides was not significant. The latter result excludes a systematic bias in positioning of the grafts or a strong tendency for axons in one dorsal column to regenerate into an ipsilateral rather than contralateral nerve segment. Sham operation or crushing of the sciatic nerve was much less effective than nerve transection in evoking spinal regeneration; after either procedure, counts of labelled neurones in ganglia on the appropriate side averaged less than 10% of those on the side from which the graft was taken. In 70 C8 DRG, the mean and range of the number of labelled neurones were 1 and 0–7, respectively. Sciatic nerve injury did not seem to influence growth into grafts of axons arising from contralateral lumbar DRG or cervical DRG.

Finally, injuries to peripheral and spinal axons were staggered in time by grafting a segment of one sciatic nerve to the spinal cord and cutting the other sciatic nerve 1 or 4 weeks earlier or later (Fig. 2b). Sciatic nerve injury was comparably effective in inducing central regeneration when it preceded rather than coincided with grafting. However, after a delay of as little as 1 week, cutting the second sciatic nerve resulted in significantly less regeneration from the corresponding dorsal column. It appeared that a propensity for regeneration persisted in sensory neurones for at least 1 month after sciatic nerve transection but that the environment near the junction between spinal cord and nerve segment became less conducive to axon outgrowth within 1 week of grafting.

After injury at high cervical level, spinal axons of neurones with cell bodies in L4 and L5 ganglia regenerated in abundance only if the ipsilateral sciatic nerve was also cut. The virtual absence of regeneration in basal conditions indicates that normal cellular function in these nerve cells does not usually support regeneration of their spinal axons injured at a distance from their somata. The enhancement of regeneration by a stimulus remote from the site of axon growth suggests that critical cellular responses affecting the entire neurone have been induced. Under other circumstances, events in these perikarya are of questionable significance during axon regeneration; for example, axons in crushed dorsal roots regrow towards the spinal cord with no obvious chromatolysis or change in axoplasmic transport^{10–12}. The neuronal reactions^{6,13,14} that promote regeneration of spinal axons, and the retrograde signals^{15–17} that trigger these cellular responses are unknown.

Priming of peripheral nerve regeneration by a preceding crush injury¹⁸ differs from the enhancement seen here in that conditioning and testing lesions are separated temporally rather than spatially. A 'pruning' effect that has been described after a single lesion to branching axons of invertebrates and vertebrates^{19–21} involves proliferation of uninjured fibres as

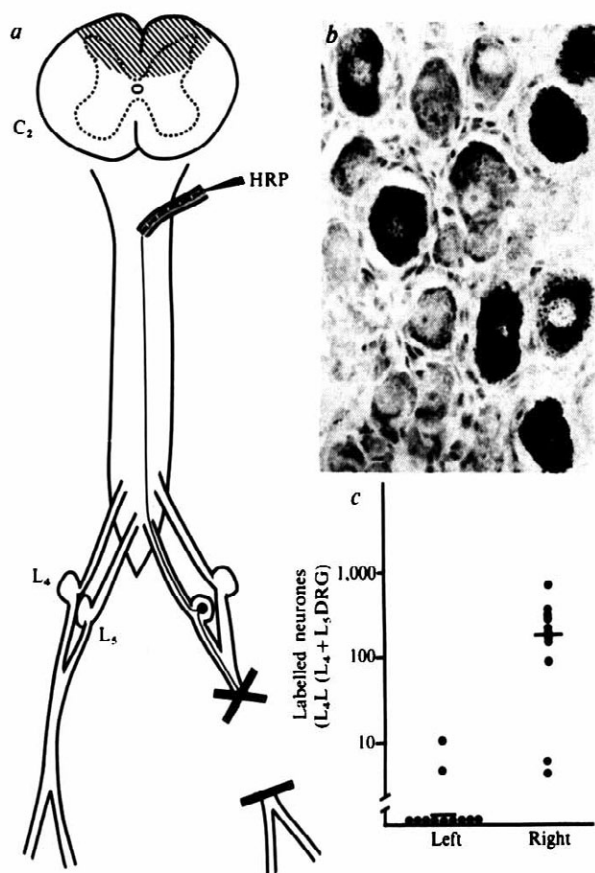


Fig. 1 *a*, Diagram showing experimental procedure. Experiments were performed on female rats weighing ~150 g and anaesthetized with pentobarbital. Under an operating microscope, the second cervical lamina was removed and a symmetrical transverse incision approximately 1 mm deep was made in the dorsal columns. One end of a 2-cm segment of the right sciatic nerve was apposed to the injured spinal cord in the midline with a stitch through the meninges. The left sciatic nerve was uninjured. Two to three months after grafting, the other end of each graft was exposed subcutaneously in the suboccipital region and injected with a total of 1 μ l 20% HRP through a glass micropipette. Two days later the rats were perfused with buffered 3% glutaraldehyde and serial sections, 20 μ m thick, were cut through the L4 and L5 DRG, reacted with tetramethylbenzidine²⁹ and counterstained with neutral red. *b*, Representative HRP-containing neurons in an L4 DRG on the right side. *c*, Labelled neurones were counted in the L4 and L5 DRG of 11 rats. To avoid double counting, only neurones with visible nucleoli were counted. The error due to split nucleoli was ignored. The difference between numbers of labelled neurones in DRG on the left and right was highly significant ($P < 0.001$) to either Mann-Whitney or Student's *t* statistical testing. Bars represent mean sums of labelled neurones in L4 and L5 DRG.

opposed to regeneration of cut axons. Possibly, all three phenomena reflect similar molecular events. Other transganglionic effects of peripheral axotomy on central axons of mammalian sensory neurones include changes in fibre diameter, conduction velocity, fast axoplasmic transport and content of peptides and enzymes^{14,22-26}. Reasons for the difference between the effects of peripheral nerve crush and transection on the function of sensory neurones^{5,23,27,28} are unclear.

Without peripheral axotomy, spinal axons of primary sensory neurones regenerate into peripheral nerve grafts no better than, for example, rubrospinal or spinocerebellar axons⁹. Very few axons from dorsal or lateral spinal tracts grow into peripheral nerve segments after injury more than a few centimetres from their cell bodies. When sensory neurones are stimulated by

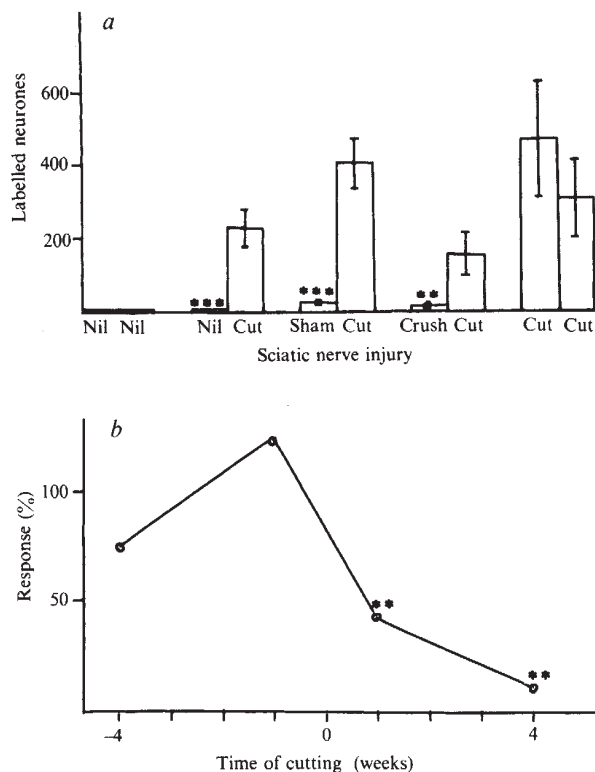


Fig. 2 *a*, Sums of labelled neurones in the L4 and L5 DRG recorded for left and right sides in five groups of rats. Nil, no operation on leg; sham, sciatic nerve exposed but not cut; cut, sciatic nerve cut at the origin of the nerve to the biceps; crush, sciatic nerve crushed for 15 s with jeweller's forceps at the level of the nerve to the biceps. Means $\pm$ s.e.m., $n = 6-11$. Asterisks indicate significantly fewer neurones than on the cut side: ** $P < 0.01$, *** $P < 0.001$ (Mann-Whitney *U*-test). *b*, In four groups of rats ($n = 6-9$), the left sciatic nerve was cut 1 or 4 weeks before or after grafting of a segment of the right sciatic nerve to the dorsal spinal cord at high cervical level. For each point, the sum of labelled neurones on the left is expressed as a percentage of the sum on the right for the entire group. ** Significant difference between the two sides ($P < 0.01$, Mann-Whitney *U*-test).

peripheral nerve transection, their spinal axons regenerate much more vigorously than without a second injury. The hypothesis that neurones lying entirely within the central nervous system might be extrinsically induced in a similar manner is exciting to contemplate but difficult to test.

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1. Björklund, A. & Stenevi, U. *Physiol. Rev.* **59**, 62-100 (1979).
2. Richardson, P. M., McGuinness, U. M. & Aguayo, A. J. *Nature* **284**, 264-265 (1980).
3. Kromer, L. F., Björklund, A. & Stenevi, U. *Brain Res.* **210**, 173-200 (1981).
4. Benfey, M. & Aguayo, A. J. *Nature* **296**, 150-152 (1982).
5. Lieberman, A. R. in *Essays on the Nervous System* (eds Bellairs, R. & Gray, E. G.) 71-105 (Clarendon, Oxford, 1974).
6. Grafstein, B. & McQuarrie, I. G. in *Neuronal Plasticity* (ed. Cotman, C. W.) 155-195 (Raven, New York, 1978).
7. Barron, K. D. in *Spinal Cord Reconstruction* (eds Kao, C. C., Bunge, R. P. & Reier, P. J.) 7-40 (Raven, New York, 1983).
8. Richardson, P. M., McGuinness, U. M. & Aguayo, A. J. *Brain Res.* **237**, 147-162 (1982).
9. Richardson, P. M., Issa, V. M. K. & Aguayo, A. J. *J. Neurocytol.* **13**, 165-182 (1984).
10. Hare, W. K. & Hinsey, J. C. *J. comp. Neurol.* **73**, 489-502 (1940).
11. Perry, G. W., Krayanek, S. R. & Wilson, D. L. *J. Neurochem.* **40**, 1590-1598 (1983).
12. Reier, P. J., Stensaas, L. J. & Guth, L. in *Spinal Cord Reconstruction* (eds Kao, C. C., Bunge, R. P. & Reier, P. J.) 163-195 (Raven, New York, 1983).
13. Skene, J. H. P. & Willard, M. J. *Cell Biol.* **89**, 96-103 (1981).
14. Perry, G. W. & Wilson, D. L. *J. Neurochem.* **37**, 1203-1217 (1981).
15. Kristensson, K. & Olsson, Y. *Brain Res.* **79**, 101-109 (1974).
16. Richardson, P. M. & Ebendal, T. *Brain Res.* **246**, 57-64 (1982).
17. Richardson, P. M. & Riopelle, R. J. *J. Neurosci.* (in the press).
18. McQuarrie, I. G. & Grafstein, B. *Archs Neurol.* **29**, 53-55 (1973).
19. Schneider, G. E. *Brain Behav. Evol.* **8**, 73-109 (1973).
20. Pickel, V. M., Segal, M. & Bloom, F. E. *J. comp. Neurol.* **155**, 43-60 (1974).
21. Scott, S. A. & Muller, K. J. *Dev Biol.* **80**, 345-363 (1980).
22. Knyihár, E. & Csillik, B. *Expl Brain Res.* **26**, 73-87 (1976).
23. Horch, K. *Brain Res.* **151**, 581-586 (1978).

24. Carlson, J., Lais, A. C. & Dyck, P. J. *J. Neuropath. exp. Neurol.* **38**, 579–585 (1979).
 25. Jessell, T., Tsunoo, A., Kanazawa, I. & Otsuka, M. *Brain Res.* **168**, 247–259 (1979).
 26. Bisby, M. A. *Neurosci. Lett.* **21**, 7–11 (1981).
 27. Barbut, D., Polak, J. M. & Wall, P. D. *Brain Res.* **205**, 289–298 (1981).
 28. Devor, M. & Wall, P. D. *J. Neurosci.* **1**, 679–684 (1981).
 29. Mesulam, M.-M. *Tracing Neural Connections with Horseradish Peroxidase* (Wiley, New York, 1982).

Vitamin E protects against retinopathy of prematurity through action on spindle cells

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In the premature infant, exposure of the incompletely vascularized retina to increased oxygen tension can result in the development of a blinding disease, retinopathy of prematurity (ROP). Despite the judicious curtailment of oxygen, the incidence of ROP is on the increase due to the technological advances that have improved the survival of the very young preterm infant¹. Six clinical trials^{2–7} have documented the efficacy of vitamin E supplementation in suppressing the development of severe ROP^{8–10}, but the mechanism of this protection has remained unknown. This report proposes that spindle cells, mesenchymal precursors of the inner retinal capillaries, are the primary inducers of the neovascularization associated with ROP. Exposure of spindle cells to elevated oxygen tension increases their gap junction area. This early morphologic event immediately halts the normal vasoformative process and eventually triggers the neovascularization that is observed clinically 8–12 weeks later. Vitamin E supplementation above the deficient plasma levels of these infants¹¹ suppresses gap junction formation and clinically reduces the severity without altering the total incidence of ROP.

The database is 69 pairs of whole-eye donations from infants who were live-born, anomaly-free at 20 to 31 weeks gestational age and ≤ 1500 g birth weight, and who survived from 30 min to 20 weeks. Twenty-two infants received minimal or no vitamin E supplementation (control infants) with mean plasma vitamin E levels of 0.3–0.6 mg%; 47 infants received vitamin E supplementation (treatment infants) with mean plasma vitamin E levels of 1.2–3.3 mg%, which are within the adult physiologic range^{12,13}. All whole eye donations contained avascular regions in all quadrants of the retina and were processed for light¹⁴, transmission¹⁵, and scanning electron microscopy. Light micrographs of continuous areas from the optic disk to the ora serrata of both the nasal and temporal hemispheres were assembled into montages. Along these montages, the following parameters were determined morphometrically: percentage of vessels with necrotic endothelial cells, percentage of spindle cells in a migrating or canalizing configuration, and percentage volume of the nerve fibre layer occupied by spindle cells¹⁴. Gap junction surface area between adjacent spindle cells was calculated from 40,000X electron micrographs using a Zeiss Videoplan and expressed as the percentage of the spindle plasma membrane surface area that was differentiated into gap junctions. All *P* values were calculated from a paired Student's *t*-test. All data are given for only the temporal hemisphere.

The morphometric analyses allow the role of the spindle cells in retinal vasoformation to be elucidated. As retinal development (Fig. 1a) and stratification (Fig. 1b) proceed peripherally from the optic disk, an apron of interdigitating spindle cells emerges from the adventitia of the hyaloid artery and migrates behind an advancing point of retinal development (Fig. 1c). The fusiform spindle cells within the migrating apron (Fig. 2a) are randomly oriented within the plane of the nerve fibre layer (Fig. 2b). Central to the fringe of the migrating apron, nascent retinal blood vessels are formed by canalization of spindle cells (Fig.

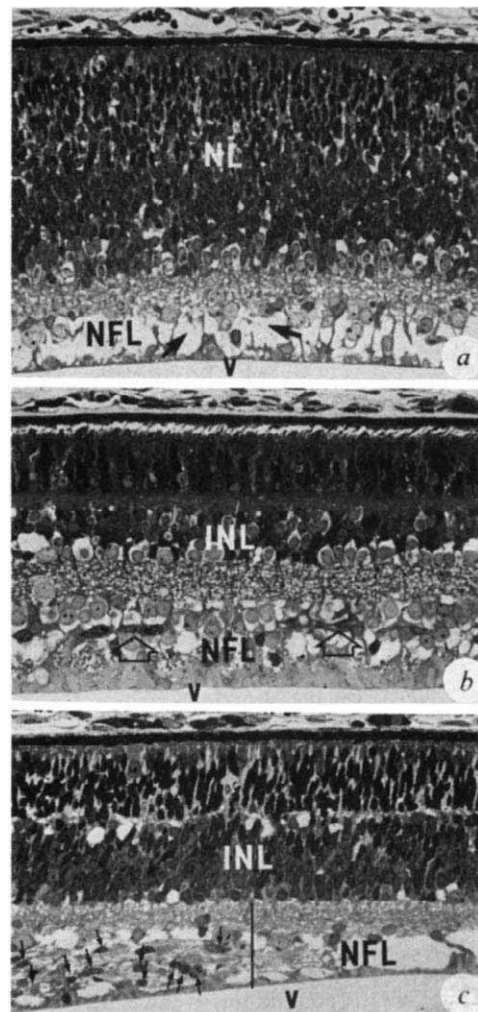


Fig. 1 Light micrographs ($\times 240$) demonstrating the relationship between the state of retinal development and the invasion of spindle cells into the nerve fibre layer. *a*, Undifferentiated midperipheral retina with primitive outer neuroblastic layer (NL) and no spindle cells within the nerve fibre layer (NFL) which is laced with cystoid spaces ($\rightarrow$). *b*, Differentiated central retina in which the nerve fibre layer (NFL) contains developing capillaries ($\diamond$). *c*, Apron of spindle cells ($\rightleftharpoons$) within the nerve fibre layer (NFL). The migration ceases at a distinct point ($\perp$) of retinal development. Inner nuclear layer (INL), vitreous (V).

2c). The canalization process involves formation of a lumen within a solid cord of spindle cells. Eventually erythrocytes from the formed capillaries penetrate the lumen (Fig. 2d). This entire sequence of migration, canalization, and endothelial differentiation normally occurs in the hypoxic ($P_{O_2} = 25$ mmHg)¹⁶ *in utero* environment.

The gap junction area of the spindle cells (Fig. 3) is modulated by oxygen tension, vitamin E supplementation, and gestational age. For the first four days post partum (14 control and 13 treatment infants), the average gap junction area remains low (4.6 ± 2.1 , range <1 –12.1) (Figs 3a, 4), with spindle cells occupying $<16\%$ of the nerve fibre layer volume. These spindle cells are in migrating and canalizing configurations. In the absence of vitamin E supplementation (8 control infants), there is a significant ($P = 0.0005$) increase in gap junction area (Fig. 3b) as early as four days post partum (Fig. 4a). There is a cessation of canalization, and severe ROP develops in this group around 9 weeks post partum^{5–7}. Despite the increase in gap junction area, the spindle cells still occupy $<13\%$ of the nerve fibre layer volume until after the development of severe ROP. The down modulation of gap junction area (>70 days post partum) (Fig. 4a) is a late event that correlates with stacking

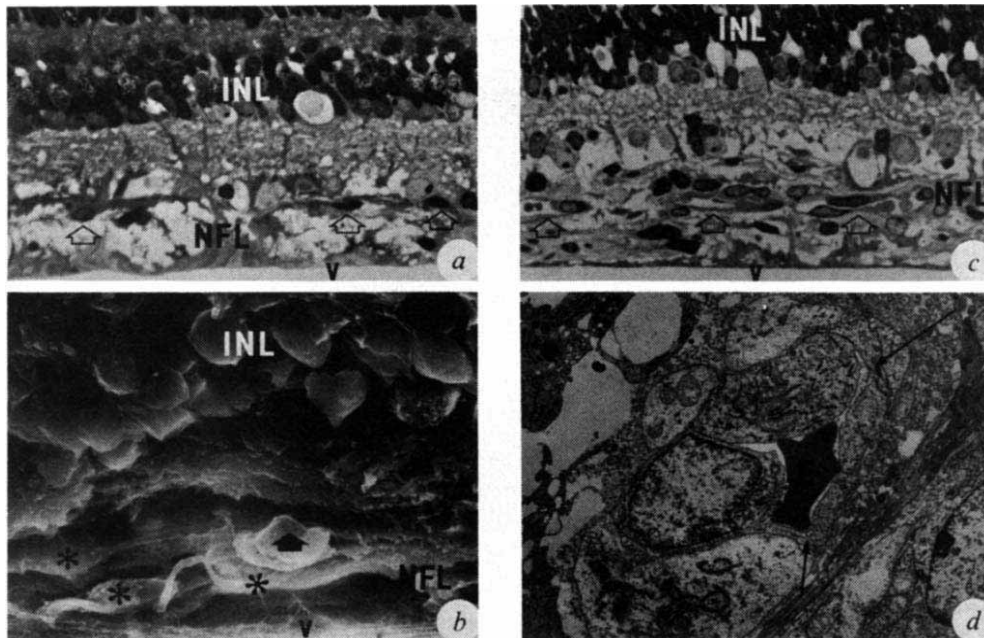


Fig. 2 Migrating and canalizing spindle cells within the developing human retina. *a*, Light micrograph ($\times 435$) of migrating spindle cells ($\blacktriangle$) forming an anastomosing apron within the nerve fibre layer (NFL). *b*, Scanning electron micrograph ($\times 2,250$) of the migrating, fusiform spindle cells which are randomly oriented within the plane of the NFL. Contrast those spindle cells lying in a plane parallel to the scanned surface ($\ast$) with those at right angles ($\blacktriangle$). Inner nuclear layer (INL), vitreous (V). *c*, Light micrograph ($\times 750$) of canalizing spindle cells ($\blacktriangle$) posterior to the advancing apron in the NFL. *d*, Electron micrograph ($\times 3,750$) of canalizing spindle cells with erythrocyte (E) penetration into the lumen and marginal flap formation ($\leftarrow$).

of the spindle cells such that they occupy 30–54% of the nerve fibre layer volume. Vitamin E supplementation suppresses gap junction formation, but this suppression is strongly dependent upon gestational age. In treatment infants ≤ 27 weeks (18 infants) (Fig. 4*b*), there is a lag period of approximately 16 days post partum in which minimal increases in gap junctions occur. Following this lag, there is a rapid increase in gap junction area to a level equivalent ($P = 0.3$) to that of unsupplemented infants. There is a parallel cessation of spindle cell canalization, but development of severe ROP in this group is delayed to around 12 weeks post partum^{4–7}. In all treatment infants shown in Fig. 4*b*, the spindle cells occupy $< 14\%$ of the nerve fibre layer volume. In treatment infants ≥ 28 weeks gestational age (Fig. 4*c*) (16 infants), gap junction formation is suppressed, canalization of spindle cells continues, spindle cells occupy a minimal volume of the nerve fibre layer ($< 13\%$), and no severe ROP develops. Thus, while continuous supplementation in infants ≥ 28 weeks gestational age suppresses gap junction formation and the development of severe ROP, supplementation in infants ≤ 27 weeks gestational age delays the onset of gap junction formation but does not diminish the severity of ROP in the highest risk infants. Vitamin E supplementation delays the down modulation of gap junction area and the clinical development of severe ROP (Fig. 4*a, b*).

Based on these ultrastructural observations, the following mechanism of the induction and suppression of ROP is proposed. When the retinas of high risk premature infants are exposed to relative hyperoxia, the elevated oxygen tension induces the formation of gap junctions between adjacent spindle cells. The gap junctions are the first morphologic evidence of oxygen toxicity, and their formation results in the subclinical cessation of spindle cell migration and canalization. The ionic coupling of spindle cells via gap junction formation changes the cells from potential endothelial precursors to sites of synthesis and release of angiogenic factors. The angiogenic factors trigger neovascularization from existing vessels over the next 8–12 weeks. Vitamin E supplementation to adult physiologic levels (1.1–3.3 mg%) suppresses gap junction formation by protecting the spindle cells from oxygen related damage, and thus reduces the severity of ROP.

This proposed mechanism of ROP induction can explain four clinical realities regarding the development, suppression, and treatment of this disease: (1) The clinical observation that

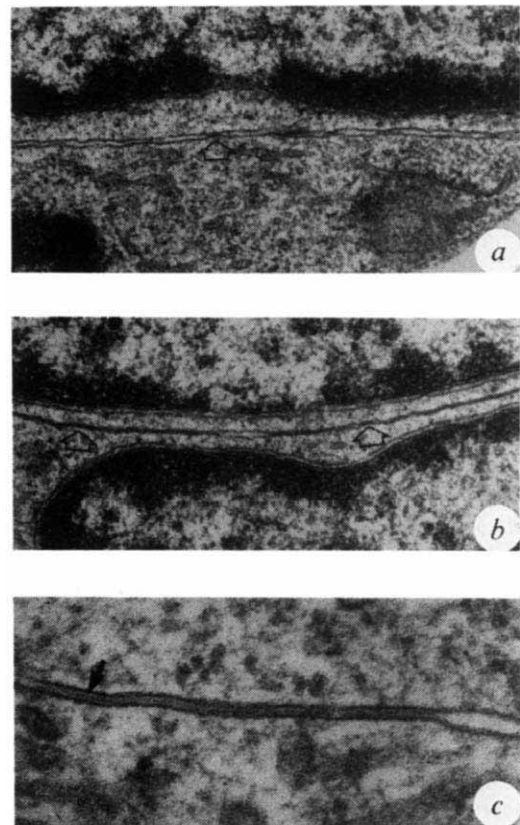


Fig. 3 Gap junctions between adjacent spindle cells. *a, b* Are electron micrographs ($\times 32,000$) demonstrating the plasma membrane surface area changes. *a*, Punctate close plasma membrane appositions ($\blacktriangle$); such spindle cells are actively migrating and canalizing. *b*, Extensive close plasma membrane appositions ($\blacktriangle$); such spindle cells have ceased migrating and canalizing and are primed to induce neovascularization from nascent capillaries. *c*, At higher magnification ($\times 110,000$), the close plasma membrane appositions are resolved as gap junctions with substructure within the 20–30 Å gap ($\rightarrow$).

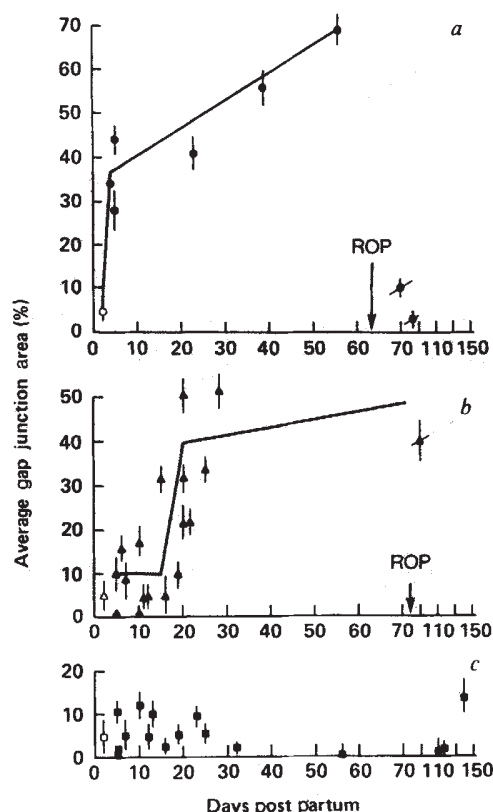


Fig. 4 Average gap junction area (%) $\pm$ s.e.m. as a function of days post partum. *a*, Control infants, $n=8$ ($\bullet$). *b*, Treatment infants ≤ 27 weeks gestational age, $n=18$ ($\blacktriangle$). *c*, Treatment infants ≥ 28 weeks gestational age, $n=16$ ($\blacksquare$). For each group, the average gap junction area in equivalent infants who survived for <4 days post partum ($\circ$, 14 infants; $\triangle$, 9 infants; and $\square$, 4 infants) is plotted. The three infants with clinically documented severe ROP are represented by $\bullet$, and $\blacktriangle$. The time when severe ROP develops for each group (arrows in *a*, *b*) is based on the results of clinical studies which enrolled 418 high risk preterm infants⁵⁻⁷.

vitamin E supplementation is not efficacious unless initiated at birth⁴⁻¹⁰ is explained by the ultrastructural observation that gap junctions can increase as early as four days post partum; thus, antioxidant protection is needed concomitantly with the increase in oxygen tension. (2) Although vitamin E supplementation suppresses gap junction increases in infants ≥ 28 weeks gestational age, it is not an absolute suppression; thus, the severe blinding grades of ROP are suppressed⁵⁻⁷ while the total incidence of all grades of ROP remains constant. (3) Severe ROP may develop in infants ≤ 27 weeks gestational age despite continuous supplementation because vitamin E only transiently suppresses increases in gap junctions in these infants. After a lag period of approximately 2 weeks, gap junctions may increase to a level commensurate with that of unsupplemented controls. This lag correlates with the delayed onset of severe ROP in the highest risk infants⁵⁻¹⁰. (4) Ablation of only the avascular peripheral retina has been utilized empirically to induce regression of severe ROP before retinal detachment¹⁷⁻¹⁹. The proposed mechanism of ROP induction would predict the success of this procedure, since it involves the destruction of the gap-junction-linked spindle cells. In contrast, ablation of the proliferating vessels or the vascular/avascular interface leads to vitreous haemorrhage and retinal separation²⁰⁻²². The ultrastructural explanation of the failure of this procedure is that the gap-junction-linked spindle cells remain operant and continue to secrete angiogenic factors and induce neovascularization.

The spindle cell mechanism for the induction of ROP contrasts with that proposed by Ashton²³ and Patz^{24,25} in which vasoconstriction, vaso-obliteration, endothelial necrosis, and hypoxia

were believed to be the initiating events. No endothelial cell necrosis was observed in any of the 69 pairs of whole eye donations in this study. Only the spindle cell concept explains the natural history of ROP and the gestational age dependence of the efficacy of vitamin E supplementation in suppressing the development of severe ROP. Furthermore, it rationalizes surgical intervention that destroys the peripheral inducers of this developmental retinopathy.

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1. Patz, A. *Ophthalmology* **90**, 425-427 (1983).
2. Johnson, L., Schaffer, D. & Boggs, T. R. *Am. J. clin. Nutr.* **27**, 1158-1173 (1974).
3. Finer, N. N., Schindler, R. F., Grant, G. D., Hill, G. B. & Peters, K. L. *Lancet* **i**, 1087-1091 (1982).
4. Finer, N. N., Schindler, R. F., Peters, K. L. & Grant, G. D. *Ophthalmology* **90**, 428-435 (1983).
5. Hittner, H. M. et al. *New Engl. J. Med.* **305**, 1365-1371 (1981).
6. Hittner, H. M. et al. *Pediatrics* **71**, 423-432 (1983).
7. Hittner, H. M. et al. *Pediatrics* **73**, 238-249 (1984).
8. Kretzer, F. L. et al. in *Developing and Regenerating Vertebrate Nervous Systems* (eds Coates, P. W., Markwald, R. R. & Kenny, A. D.) 199-210 (Liss, New York, 1983).
9. Hittner, H. M. & Kretzer, F. L. *Ciba Fdn Symp.* **101**, 165-185 (1983).
10. Hittner, H. M., Kretzer, F. L. & Rudolph, A. J. *Hos. Pract.* **19**, 85-99 (1984).
11. Farrell, P. M. J. *Pediatr.* **95**, 869-872 (1979).
12. Sobel, S., Gueriguian, J., Troendle, G. & Nevius, E. *New Engl. J. Med.* **306**, 867 (1982).
13. Hittner, H. M. et al. *New Engl. J. Med.* **309**, 669-670 (1983).
14. Kretzer, F. L., Hittner, H. M., Johnson, A. T., Mehta, R. S. & Godio, L. B. *Ann. N. Y. Acad. Sci.* **393**, 145-166 (1982).
15. Kretzer, F. L., Hittner, H. M. & Mehta, R. S. *Arch. Ophthalmol.* **99**, 2000-2006 (1981).
16. Flower, R. W. & Blake, D. A. *Pediatr. Res.* **15**, 1293-1302 (1981).
17. Ben Sira, I., Nissenkorn, I., Grunwald, E. & Yassur, Y. *Br. J. Ophthalmol.* **64**, 758-762 (1980).
18. Mousel, D. K. & Hoyt, C. S. *Ophthalmology* **11**, 1121-1127 (1980).
19. Nissenkorn, I., Kremer, I., Ben Sira, I., Cohen, S. & Garner, A. *Br. J. Ophthalmol.* **68**, 36-41 (1984).
20. Flynn, J. T. *Trans. Am. Acad. Ophthalmol. Otolaryngol.* **76**, 1234-1246 (1972).
21. O'Grady, G. E., Flynn, J. T. & Herrera, J. A. *South. med. J.* **65**, 655-658 (1972).
22. Kingham, J. D. *Arch. Ophthalmol.* **96**, 2049-2053 (1978).
23. Ashton, N. *Br. med. Bull.* **26**, 103-106 (1970).
24. Patz, A. *Curr. Eye Res.* **3**, 159-163 (1984).
25. Patz, A. *Br. J. Ophthalmol.* **68**, 42-46 (1984).

Correlation between inositol phospholipid hydrolysis and substance P receptors in rat CNS

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The undecapeptide substance P is a neurotransmitter candidate in the mammalian central and peripheral nervous system¹. Although the distribution of substance P-like immunoreactivity within the central nervous system (CNS) is well established², the recent identification and autoradiographic localization of specific substance P-binding sites has revealed numerous areas of mismatch between peptide levels and numbers of such sites³⁻⁶. Previous studies have shown that substance P stimulates the hydrolysis of inositol phospholipids in peripheral tissues and in the hypothalamus^{7,8}, probably through stimulation of a polyphosphoinositide-specific phospholipase C (refs 9-11). Inositol phospholipid hydrolysis has been implicated in the mobilization of cytosolic calcium following receptor activation in several neurotransmitter and hormonal systems¹². We have therefore investigated the distribution of ³H-labelled substance P binding sites within various rat brain regions and correlated this with the rate of substance P-induced hydrolysis of inositol phospholipids in the same areas of the CNS. We found that the rate of inositol phospholipid hydrolysis was proportional to the number of binding sites specific for ³H-substance P, suggesting that binding sites revealed by ³H-substance P autoradiography correspond to functional substance P receptors.

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The distribution of specific ^3H -substance P-binding sites throughout the rat brain was initially determined. Adult male Sprague-Dawley rats were killed, their brains removed, frozen on dry-ice and 20- μm sections cut using a cryostat in either the horizontal or the coronal plane. They were apposed onto gelatin-

ized glass slides and stored in boxes with desiccant for 48 h at -20°C . The sections were then preincubated at room temperature for 10 min in a solution containing 0.005% polyethyleneimine in 50 mM Tris-HCl, pH 7.40, followed by incubation in the presence of ^3H -substance P. Nonspecific binding was

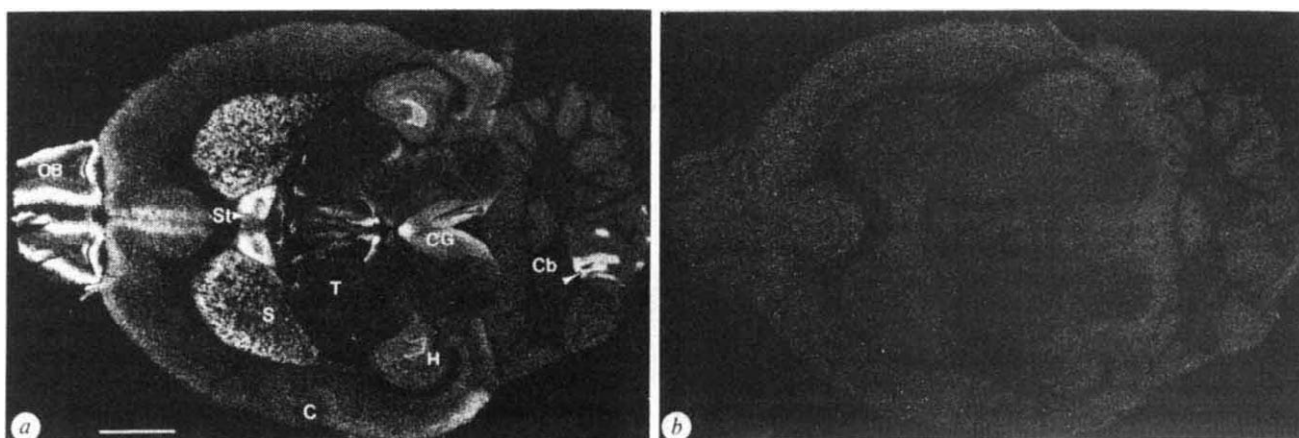
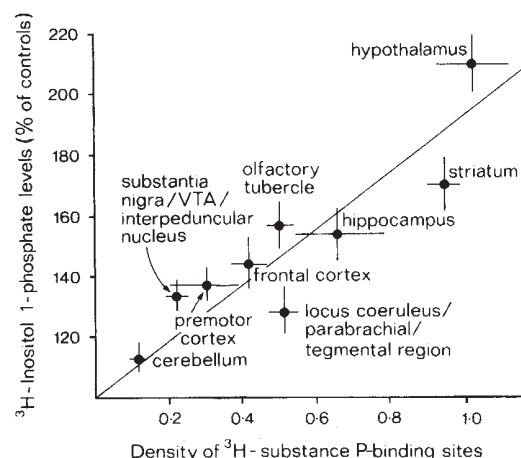


Fig. 1 ^3H -substance P-receptor distribution in the rat brain. *a*, A typical darkfield photomicrograph of ^3H -substance P binding in a horizontal brain section. The light areas are silver grain concentrations indicative of specific binding. *b*, A darkfield photomicrograph of an adjacent control section treated identically to *a*, except that 1 μM substance P (Peninsula) was added to the incubation solution. Note the heavy labelling in several areas, such as the external plexiform layer of the olfactory bulb (OB), the striatum (S), the septum (St), the central grey (CG) of the midbrain and folia IX and X of the cerebellum (Cb, arrowhead), moderate labelling in the cortex (C) and hippocampus (H) and absence of label in the thalamus (T). Scale bar, 2.5 mm.

Methods: Horizontal 20- μm sections were preincubated at room temperature for 10 min in a solution of 50 mM Tris-HCl (pH 7.4) containing 0.005% polyethyleneimine (Sigma). The slides were then incubated for 10 min in a solution of 50 mM Tris-HCl (pH 7.4) containing 0.02% bovine serum albumin (Sigma, radioimmunoassay grade), chymostatin (2 mg l^{-1} ; Sigma), leupeptin (4 mg l^{-1} ; Sigma), bacitracin (40 mg l^{-1} ; Sigma) and increasing concentrations of [2-L-prolyl-3,4- ^3H (N)] substance P (specific activity 26 Ci mmol^{-1} , NEN). Nonspecific binding was defined as binding in the presence of 1 μM substance P (Peninsula) and specific binding obtained by subtracting the nonspecific binding from the total binding. In these incubation conditions >95% of ^3H -substance P eluted at the position of intact substance P on reverse-phase HPLC and the specific binding reached a plateau after about 8 min. The slides were rinsed with four washes of ice-cold 50 mM Tris-HCl (pH 7.4), 5 s each, and four washes of water, 5 s each, dried in a stream of cold air and stored overnight in desiccant. The slides were then placed in apposition to a tritium-sensitive film (^3H -Ultrofilm, LKB). After 12 weeks the film was developed using Kodak D-19 developer and photographed under darkfield illumination. To estimate the relative density of specific ^3H -substance P-binding sites we used microdensitometry combined with the tritium-sensitive film, as described previously¹⁴. The exposed film was placed on the stage of a Leitz orthoplan microscope equipped with a Vario-Orthomat camera system. Direct reading of the voltage from the photosensitive cell served as a density reading. Calibration experiments were used to determine in which region the density was linearly dependent on the isotope concentration and the measurements were performed in that region. The densitometer was able to resolve density differences of the order of 0.02 A units and readings were taken from an area of 200 μm diameter. Background labelling was defined as the ^3H -substance P binding in the presence of 1 μM substance P.

Fig. 2 The density of substance P receptors plotted against the magnitude of substance P-induced inositol phospholipid hydrolysis in various regions of the rat brain. The density of substance P receptors was determined using autoradiography coupled to a tritium-sensitive film and analysed using microdensitometry, as described for Fig. 1. All readings were taken from sections incubated in and exposed to the same conditions. To quantify the relative density of receptors, we designated the highest area measured (the hypothalamus) as 1.0 and ranked the others accordingly. The mean $\pm$ s.e.m. of at least four determinations throughout the area under examination was taken.

Methods: The substance P-induced hydrolysis of inositol phospholipids in various regions of the rat central nervous system was determined according to Berridge *et al.*¹⁵. Sprague-Dawley rats (150–200 g) were killed, the brain removed, blocked and placed into an oxygenated solution of artificial cerebrospinal fluid (CSF, composition in mM: NaCl, 124; KCl, 2; KH_2PO_4 , 1.25; MgSO_4 , 2; NaHCO_3 , 25 and glucose, 11). Sections (300 μm) were cut using a vibratome (Oxford Instruments) and the appropriate brain area dissected out using microscissors; one side was used for stimulation with substance P and the contralateral side served as the control. Sections were placed in CSF solution for 2 h at 37°C and then transferred to 500 μl of prewarmed oxygenated CSF to which 1 μCi myo-[2- ^3H]inositol (specific activity 10–20 Ci mmol^{-1}) (Amersham) and 12 mM LiCl had been added. One hour later 1 μM substance P (Peninsula) was added and the tubes were incubated for another 30 min. The incubation was terminated by addition of 500 μl trichloroacetic acid and the tubes were left for 15 min on ice. Following a 15-min centrifugation at 2,000g, 700 μl of the supernatant was removed. The latter was then washed three times with 4 ml of ether, with the ether phase being discarded after each wash, and 700 μl of 20 mM Tris-HCl containing 5 mM EDTA was added to each tube. Tritiated inositol 1-phosphate was separated from other water-soluble metabolites by application of the sample to columns containing 1 ml of Dowex 1-X8 (Sigma) in the formate form. The columns were washed twice with 8 ml of 60 mM ammonium formate/5 mM disodium tetraborate and ^3H -inositol 1-phosphate was eluted with 8 ml of 200 mM ammonium formate/100 mM formic acid and the radioactivity determined by liquid scintillation spectrometry. The levels of ^3H -inositol 1-phosphate are expressed as per cent of controls (taken as 100). In a typical experiment on the hypothalamus the control value was $1,844 \pm 127$ d.p.m. ^3H -inositol 1-phosphate per tissue slice ($n = 6$). The results are expressed as the means $\pm$ s.e.m. of triplicate determinations from four separate experiments.



defined in the presence of 1 μ M substance P. Preliminary experiments had shown that, in the absence of polyethyleneimine, >95% of the 3 H-substance P binding was to the glass around the tissue rather than to the tissue itself, while specific binding reproducibly amounted to 60% of the total binding in the presence of polyethyleneimine. Following incubation in the presence of 3 H-substance P, tissue sections were apposed to a tritium-sensitive film.

Previous experiments¹³ had shown that 3 H-substance P bound to a single class of binding sites with an equilibrium dissociation constant of 1.1 nM and a maximum number of binding sites of 15 fmol per mg tissue. The fragment potencies agreed well with results from studies on the CNS which had used tissue homogenate binding assays for substance P^{4,5}.

The correlation between the autoradiographic distribution of specific 3 H-substance P-binding sites (Fig. 1) and a biochemical response linked to substance P-receptor occupancy was investi-

gated by incubating vibratome sections from various brain regions in the presence of 1 μ M substance P and measuring the rate of inositol phospholipid hydrolysis. When the magnitude of substance P-stimulated inositol phospholipid hydrolysis was plotted against the mean number of specific substance P-binding sites in various brain regions, an excellent correlation was obtained (correlation coefficient 0.96, as judged by regression analysis; Fig. 2).

The present results therefore indicate that, while the distribution of substance P receptors in the CNS does not correlate with the distribution of substance P-like immunoreactivity³⁻⁶, the increase in inositol phospholipid hydrolysis effected by substance P is directly proportional to the number of specific 3 H-substance P-binding sites present in various brain regions. Given the interest in substance P agonists and antagonists, this measure of inositol phospholipid hydrolysis should provide a reliable biochemical assay for substance P receptors in the CNS.

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- Jessell, T. M. *Handbk Psychopharmac.* 16, 1-105 (1983).
- Ljungdahl, A., Hökfelt, T. & Nilsson, G. *Neuroscience* 3, 861-943 (1978).
- Cascieri, M. A. & Liang, T. J. *biol. Chem.* 258, 5158-5164 (1983).
- Torrens, Y., Beaujouan, J. C., Viger, A. & Glowinski, J. *Naunyn-Schmiedeberg's Archs Pharmac.* 324, 134-139 (1983).
- Viger, A., Beaujouan, J. C., Torrens, Y. & Glowinski, J. *J. Neurochem.* 40, 1030-1039 (1983).
- Quirion, R. *et al. Nature* 303, 714-716 (1983).

- Hanley, M. R., Lee, C. M., Jones, L. M. & Mitchell, R. H. *Molec. Pharmac.* 18, 78-83 (1980).
- Watson, S. P. & Downes, C. P. *Eur. J. Pharmac.* 93, 245-253 (1983).
- Creba, J. A. *et al. Biochem. J.* 212, 733-747 (1983).
- Berridge, M. J. *Biochem. J.* 212, 849-858 (1983).
- Downes, C. P. & Wusteman, M. M. *Biochem. J.* 216, 633-640 (1983).
- Berridge, M. J. *Biochem. J.* 220, 345-360 (1984).
- Mantyh, P. W., Maggio, J. E. & Hunt, S. P. *Brain Res.* (in the press).
- Hunt, S. P. & Mantyh, P. W. *Brain Res.* 291, 203-217 (1984).
- Berridge, M. J., Downes, C. P. & Hanley, M. R. *Biochem. J.* 206, 287-294 (1982).

H-2-linked low-molecular weight polypeptide antigens assemble into an unusual macromolecular complex

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The major histocompatibility complex (MHC) is a cluster of tightly linked genes whose products are of central importance in the functioning of the immune system. Class I and II MHC antigens are integral membrane proteins which regulate cell-surface interactions between T cells and their targets¹⁻³, while class III antigens are components of the complement system of serum proteins⁴. All available evidence indicates that the structure and function of the MHC and its gene products are highly conserved among species (for review, see ref. 5). We recently reported⁶ the existence in murine cells of a fourth class of MHC-linked polypeptides which are biochemically and genetically distinct from previously identified MHC gene products: BALB.B anti-BALB/c (anti-H-2^d) antiserum immunoprecipitates a set of 16 cytoplasmic low-molecular weight polypeptides (LMP) from BALB/c spleen cells and from the WEHI-3 cell line. The production of these peptides is coordinately regulated (by immune interferon) with the production of the class I and II MHC antigens^{7,8}, suggesting that they too are functionally relevant to the immune system. We demonstrate here that these 16 polypeptides are associated with one another *in vivo* as a very large (580,000-molecular weight, M_r) noncovalent complex. The unusual nature of this complex has allowed the non-immunochemical identification of similar complexes from (serologically negative) H-2^b murine cells and from a human cell line. Thus, LMP antigens display two properties in common with other MHC antigens: they are both polymorphic and genetically conserved across species.

To determine whether our antiserum recognizes 16 different chains, or whether some of the LMP are associated with one another *in vivo*, extracts of 35 S-methionine-labelled WEHI-3 cells were size-fractionated by gel filtration before immunoprecipitation. As Fig. 1 shows, all of the LMP activity elutes from the column as a single peak of $\sim 580,000 M_r$. No LMP activity is detectable in the lower molecular weight column fractions. This peak of LMP activity is clearly distinct from the void volume peak (Fig. 1) which, in the absence of detergent, contains all of the (aggregated) H-2 and Ia antigens present in

the original sample (data not shown), and presumably any other nonspecifically aggregated material. More importantly, the LMP peak is bounded on both sides by molecular weight markers. The results of gel filtration using gels of different exclusion limits and with different buffers (Table 1) were always consistent with the data shown in Fig. 1. This experiment therefore strongly suggests that all 16 LMP polypeptides are specifically associated with one another *in vivo* in the form of a high-molecular weight complex.

Figure 2b, c compares the peak LMP fraction from the gel filtration column with an LMP immunoprecipitate, and shows that nearly all of the peptides found on the portion of the gel shown ($M_r \approx 12,000$ -35,000) in Fig. 2c are LMP chains. Note, however, that there is a large amount of labelled material in the higher molecular weight portion ($M_r > 50,000$; not shown) of the gel in Fig. 2c which does not appear in LMP immunoprecipitates and is therefore not part of the LMP complex. In fact, the purity of the peak chromatography fraction can be roughly estimated by comparing the total number of 35 S.c.p.m. in the fraction with the number of c.p.m. in the LMP chains (determined by immunoprecipitation). By this criterion, the LMP comprise only approximately 1-2% of the total material in the fraction.

Thus, a single step of gel filtration yields a sample of LMP which is sufficiently pure to be visualized cleanly by two-dimensional polyacrylamide gel electrophoresis (PAGE) without prior immunoprecipitation. We have also used this technique to analyse the remaining column fractions in Fig. 1 (data not shown). No LMP chains are detectable in any of the fractions except that corresponding to the LMP peak shown, indicating that the individual LMP chains do not exist in an uncomplexed form. In addition, for all of the fractions in which LMP chains are detectable, the relative intensity of the individual chains seems to be constant. These experiments therefore suggest

Table 1 Gel filtration of LMP complexes in various conditions

Sephacryl S200	TBS + 0.5% NP40	>250,000*
Sephacryl S200	DPBS	>250,000*
Sephacryl S200	DPBS + 0.5 M NaCl	>250,000*
Sepharose CL-6B	DPBS	$\approx 580,000$
Sepharose CL-6B	DPBS + 0.5 M NaCl	$\approx 580,000$

TBS, Tris-buffered saline; DPBS, Dulbecco's phosphate-buffered saline (Gibco); NP40, Nonidet P-40.

* All LMP activity was present in the column void volume peak.

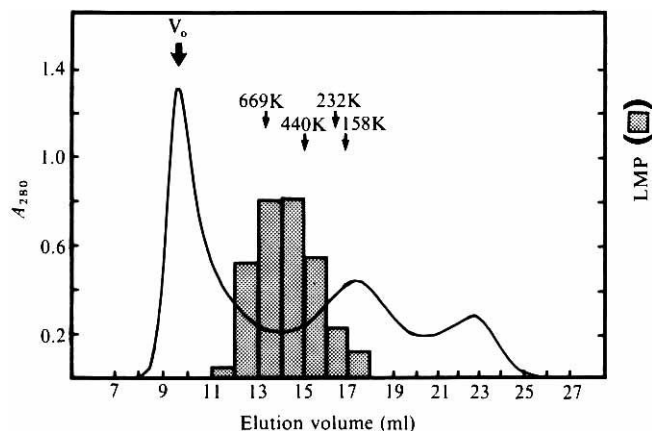


Fig. 1 Immunoprecipitation of LMP antigens from size-fractionated WEHI-3 extracts. Cells were grown and labelled with ^{35}S -methionine as described previously⁶. Before labelling, cells were grown for 2–4 days in the presence of concanavalin A-activated spleen cell supernatants to induce maximal LMP expression (see ref. 8). For gel filtration experiments, the washed labelled cells were resuspended in Dulbecco's phosphate-buffered saline (Gibco) containing 0.1% sodium azide (DPBS) and lysed by five cycles of freeze/thaw. Membranes and insoluble cellular debris were pelleted by centrifugation, and the clear supernatants were stored at -70°C until use. A 0.5×30 cm Sepharose CL-6B column calibrated with molecular weight standards (Pharmacia) was used. Arrows designate the elution volume of molecular weight markers: V_0 , void volume; 669K, thyroglobulin; 440K, ferritin; 232K, catalase; 158K, aldolase. Samples (0.5 ml) were run in DPBS or DPBS containing 0.5 M NaCl, and 1-ml fractions were collected. Immunoprecipitations were performed on column fractions as described previously⁶, except that 500 μl of sample and 50 μl of antiserum were used. For samples to be analysed without immunoprecipitation, 10 μl of sample were mixed with 10 mg of urea and 10 μl of isoelectric focusing sample buffer; 20 μl of this mixture were loaded onto the gel. Two-dimensional PAGE was performed as described previously⁶. The amount of LMP antigens was measured by densitometry of the resulting autoradiographs, and is reported in arbitrary units.

the existence of a single LMP complex with a fixed stoichiometric ratio of subunits, rather than several types of complexes containing different ratios of the individual subunits and differing slightly in molecular size. However, it is formally possible that, in addition to the protein subunits already identified, non- ^{35}S -methionine-labelled material (carbohydrates or peptidoglycans) are present in the LMP complex. In this respect, it may be significant that western blotting experiments have failed to reveal any such components (unpublished observations), indicating that if they do exist, they are probably not antigenic. When considered with the protein polymorphism data presented below, which indicate that only one of the LMP chains is polymorphic (and thus potentially antigenic) in the strain combinations used, these results argue strongly for a single complex containing all the LMP chains.

The antiserum we originally used to identify the LMP antigens was BALB.B ($H-2^b$) anti-BALB/c ($H-2^d$), which detects LMP antigens in the *a*, *d*, *f*, *ja*, *k*, *s*, *u*, *v* and *z* haplotypes. However, antisera made in the *d*, *k* or *s* haplotypes against the $H-2^b$ haplotype fail to precipitate LMP-like polypeptides from $H-2^b$ haplotype spleen cells or cell lines (J.J.M. & H.O.M., unpublished observations). In considering the possible function of the LMP antigens, it is important to decide between two possible explanations for this observation: (1) no LMP antigens are made in *b* haplotype mice or (2) although *b* haplotype mice synthesize LMP polypeptides, these are not immunogenic in *d*, *k* or *s* haplotype mice.

We can choose between these alternatives by using a non-immunochemical method of visualizing LMP antigens. Figure 2*d* shows the ^{35}S -labelled extract from the ($H-2^b$) IC21 cell line⁹ manipulated in a manner identical to the WEHI-3 extract shown in Fig. 2*c*. At least 13 of the 16 LMP chains found in

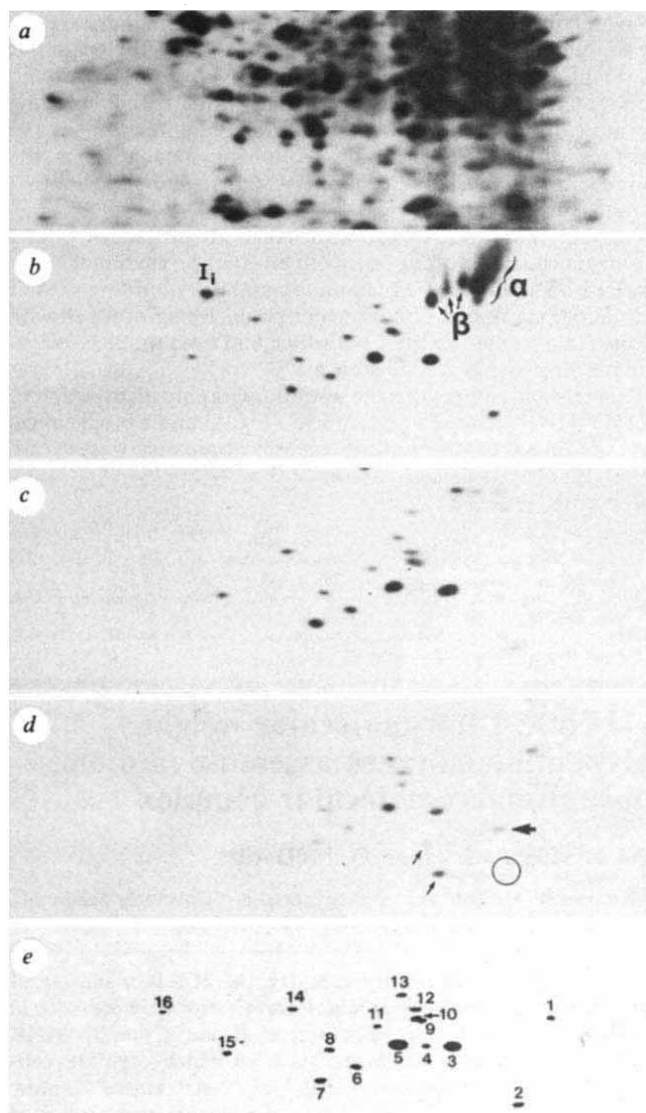


Fig. 2 Two-dimensional PAGE analysis of LMP antigens from unfractionated WEHI-3 extract (*a*), anti- $H-2^d$ immunoprecipitate of WEHI-3 extract (*b*), peak LMP fraction (fraction 14, not immunoprecipitated) from Sepharose CL-6B column of WEHI-3 (*c*) and fraction 14 from the same column, but using IC21 extract instead of WEHI-3 (*d*). LMP chains are numbered in the schematic diagram (*e*) of the $H-2^d$ haplotype pattern. Ia antigen α , β and invariant (I_i) chains are present in the immunoprecipitate (*b*) but not in the column fractions. The circle in *d* denotes the position of LMP chain 2, which seems to be absent from the *b* haplotype; the heavy arrow indicates its putative allelic form. Peptides denoted by the light arrows in *d* are probably not LMP chains, as they are also present in $H-2^k$ haplotype column fractions (see Fig. 3*a*), but are not co-precipitated with the LMP complex from those cells (not shown).

($H-2^d$) WEHI-3 cells are also found in the IC21 cell line. LMP chains 4 and 16 are probably too weak to be seen in the IC21 gel (note that LMP chain 16 is also too weak to be seen in the immunoprecipitate shown in Fig. 2*b*). Thus, only one of the LMP chains found in WEHI-3 is clearly missing from the IC21 gel (LMP 2), and IC21 contains an extra chain which is not present in WEHI-3 (heavy arrow in Fig. 2*d*). Although definitive identification of the extra chain in the IC21 gel as part of the LMP complex will require either immunoprecipitation of the complex or peptide mapping/sequencing, this experiment strongly suggests that mice of the $H-2^b$ haplotype produce an LMP complex similar to that in mice of other $H-2$ haplotypes.

The above results are also consistent with the conclusion that the LMP are associated into a large complex; an apparent

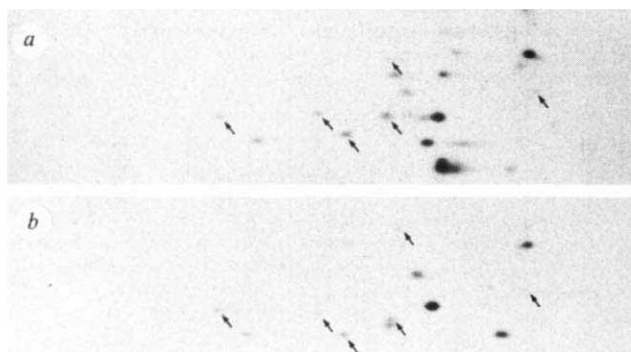


Fig. 3 Two-dimensional PAGE comparison of peak LMP column fractions (not immunoprecipitated) from the murine $H-2^k$ cell line UNC2 (a) and the human cell line, U937 (b). Chains which migrate to identical positions are denoted by arrows.

difference in only one of the peptides between the $H-2^b$ and $H-2^d$ haplotypes is sufficient to allow the production of an antibody which precipitates all 16.

As the gene products of the MHC have been highly conserved throughout evolution⁵, one might expect that complexes similar to that described here would also be present in other species. The biochemical purification procedure described above might therefore allow the identification of such complexes in the absence of a specific precipitating antibody. Figure 3 shows a two-dimensional PAGE comparison of the peak LMP fraction from a murine cell line (UNC2, $H-2^k$)¹⁰ and the corresponding fraction from the extract of a human cell line (U937)¹¹. Although the patterns are not identical, the total number of chains present in this portion of the gel is similar, and at least six of the peptides from the human cell line migrate to exactly the same position as the corresponding murine LMP (arrows). It therefore seems likely that human cells do indeed contain a complex similar to the murine LMP complex. Definitive identification of these peptides as homologues of the murine LMP, and their genetic mapping, will, however, require further investigation.

Thus, both murine and human cells contain a large molecular complex composed of approximately 16 different polypeptide chains, all of which have a molecular weight of between 15,000 and 30,000. The unusual nature of this complex is highlighted by the fact that (non-immunoprecipitated) size-fractionated extracts can be made which, when electrophoresed in denaturing SDS gels, are seen to contain only LMP chains in the low-molecular weight portion of the gel. Thus, few or no other molecular species of similar size (M_r 500,000–650,000) contain any peptides of 15,000–30,000 M_r , whereas the LMP complex is composed entirely of subunits in this size range. (Although we have presented evidence elsewhere⁶ that the multiple LMP chains are not simply proteolytic breakdown products of one or more larger molecule(s), we are currently attempting to verify this by characterizing the size of the messenger RNAs encoding the individual LMP chains.) In addition, excluding the ribosome (which has a molecular weight of $4-5 \times 10^6$ and may contain up to 80 different proteins¹²), we are unaware of any other biological macromolecule composed of so many different polypeptide chains. Thus, the LMP complex represents a unique macromolecular structure. That this unique structure is apparently conserved among species suggests that it is required for the proper functioning of the LMP complex. Experiments aimed at gaining insight into the nature of this function are underway.

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- Zinkernagel, R. M. & Doherty, P. C. *Adv. Immun.* **27**, 51–177 (1980).
- Rosenthal, A. S. *Immun. Rev.* **40**, 136–152 (1978).
- Klein, J., Juretic, A., Baxevanis, C. N. & Nagy, Z. A. *Nature* **291**, 455–460 (1981).
- Shreffler, D. C. *Transplant. Rev.* **32**, 140–167 (1976).
- Goetz, D. (ed.) *The Major Histocompatibility System in Man and Animals* (Springer, Berlin, 1977).
- Monaco, J. J. & McDevitt, H. O. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3001–3005 (1982).
- Monaco, J. J., Ku, G. & McDevitt, H. O. in *Ir Genes: Past, Present and Future* (eds Pierce, C. W., Cullen, S. E., Kapp, J. A., Schwartz, B. D. & Shreffler, D. C.) 69–73 (Humana, Clifton, New Jersey, 1983).
- Monaco, J. J. & McDevitt, H. O. *J. Immun.* (submitted).
- Mauel, J. & Defendi, V. *J. exp. Med.* **134**, 335–350 (1971).
- Lanier, L. L. *et al. Immunogenetics* **16**, 367–371 (1982).
- Ralph, P., Moore, M. A. S. & Nilsson, K. *J. exp. Med.* **143**, 1528–1533 (1976).
- Wool, T. G. A. *Rev. Biochem.* **48**, 719–754 (1979).

Role of γ -interferon in antibody-producing responses

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Interferon preparations, especially those containing γ -interferon (IFN- γ), have long been known to modulate immune responses^{1–3}. However, because many studies used only partially purified interferons, it has been difficult to separate the immunoregulatory effects of the interferons from those of other biologically active molecules contaminating the preparations. Recently, with the cloning of the interferon genes in mouse and man, it has become possible to use these cloned interferons directly to test their effects in assays other than those involving the protection of cells from viruses. For example, cloned IFN- γ has been shown to be a potent inducer of Ia antigen expression on macrophages^{4,5}. Similarly, cloned IFN- γ has been reported to act as a macrophage activation factor, as judged by the ability of activated macrophages to kill tumour cells *in vitro*⁶. We demonstrate here that cloned murine IFN- γ can also substitute for a late-acting helper factor which acts synergistically with other helper factors in the stimulation of B-cell antibody responses *in vitro*.

We have previously described⁷ a murine T-cell hybridoma, FS7-20, which on stimulation with concanavalin A (Con A) produced at least five lymphokine activities: IFN- γ , macrophage activation factor (MAF), Ia-inducing factor (IaIF), a B-cell helper factor which we termed interleukin-X (IL-X), and interleukin-2 (IL-2). In studying a large panel of clones and subclones of FS7-20, we observed a random segregation of the ability to produce IL-2 from the ability to produce the other four lymphokine activities. On the other hand, IFN- γ , MAF, IaIF and IL-X production were all tightly linked among these clones and subclones. We suggested that these four activities were probably due to the same molecular species, but we could not definitely rule out other possible explanations for the linkage or that some other common genetic control of production existed which varied among the clones and subclones of FS7-20. It had been suggested, and was later confirmed using cloned IFN- γ , that IFN- γ might act as IaIF and MAF^{4,8}, but an identity between IFN and IL-X was unexpected. Therefore, with the availability of cloned IFN- γ , we were particularly interested in testing its activity as a B-cell helper factor.

Our previous studies^{9,10} had demonstrated that the optimal *in vitro* antibody responses of highly purified murine B cells to antigens such as sheep red blood cells (SRBC) required at least three different helper factors. The first was contained in preparations from normal macrophages or from the macrophage tumour cell line P388D1. The activity in these preparations has been indistinguishable from interleukin-1 (IL-1). The second factor preparation was the concentrated Con A-stimulated supernatant of the murine T-cell hybridoma, FS6-14.13 (ref. 11). Thus far the only known essential activity in this preparation has been IL-2 (refs 9, 10), although it remains possible that other as yet unidentified molecules in this supernatant contribute to

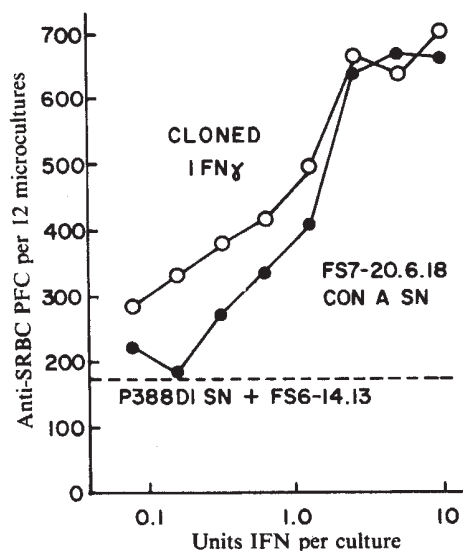


Fig. 1 IFN- γ is a B-cell helper factor. Data shown here are the sum of the anti-SRBC PFC obtained in 12 identical microculture wells of B cells incubated with P388D1 and FS6-14.13 supernatant (SN) clones (---) or with these supernatants and different numbers of units of IFN- γ contained in supernatants of the T-cell hybridoma FS6-20.6.18 (●) or derived from the cloned gene (○). **Methods:** Splenic B cells from (C57BL/6 $\times$ DBA/2) F_1 mice were purified by treatment *in vivo* with rabbit anti-mouse thymocyte serum (Microbiological Associates), and *in vitro* passage over Sephadex G-10 (Pharmacia) to remove macrophages, followed by treatment with a cocktail of anti-T-cell reagents and rabbit complement⁹⁻¹¹. These B cells were incubated in 0.1-ml microcultures in limiting numbers (10^5 per well) with 0.005% SRBC and saturating concentrations of P388D1 supernatant (20%) as a source of IL-1 and 1 μ l of a 50 $\times$ concentrated supernatant from FS6-14.13 (containing 50 U μ l IL-2) as a source of IL-2 and B-cell growth factor. 20 μ l of supernatants containing different concentrations of IFN- γ were added at the optimal time, that is, after 24 h of culture. Supernatants were either dilutions of those induced by Con A treatment of the T-cell hybridoma FS7-20.6.18 (ref. 7), or dilutions of IFN- γ derived from the cloned gene^{12,13}. The cloned gene was provided as a product purified from *E. coli* containing an expression vector that contained murine IFN- γ cDNA (specific antiviral activity of 8×10^6 U per mg protein)^{12,13}. After 4 days' culture, wells were assayed for direct PFC against SRBC by standard techniques⁹⁻¹¹.

its activity. The third required helper factor, which we termed IL-X because it did not correspond to any well-defined known molecule, was originally found in the 30,000–50,000 molecular weight fraction of an IL-2-depleted, Con A-stimulated supernatant of normal spleen cells. Its activity was assayed by its ability to enhance the antibody response of B cells to SRBC in cultures containing saturating amounts of IL-1 and IL-2 preparations. It was this activity which we later found in the Con A-stimulated supernatant of FS7-20 and whose production correlated strongly with that of IFN- γ .

To test directly whether IFN- γ could act as IL-X, we compared cloned IFN- γ produced in *Escherichia coli*^{12,13} with a standard preparation of Con A-stimulated supernatant from FS7-20.6.18, a subclone of FS7-20 which produced high levels of IFN- γ and IL-X, but barely detectable levels of IL-2. Murine B cells were cultured for 4 days with SRBC, saturating amounts of IL-1 and IL-2 preparations and various concentrations of either cloned IFN- γ or the FS7-20.6.18 supernatant. Cultures were then assayed for anti-SRBC plaque-forming cells (PFC). The results are shown in Fig. 1. As we have reported previously¹⁰, the combination of IL-1 and IL-2 stimulated a small anti-SRBC response from the B cells which was increased by addition of the FS7-20.6.18 supernatant (Fig. 1). The cloned IFN- γ produced a similar increase in the response (Fig. 1). For both preparations, the number of units of IFN- γ added predicted their relative activity in the PFC assay. Fifty per cent of the

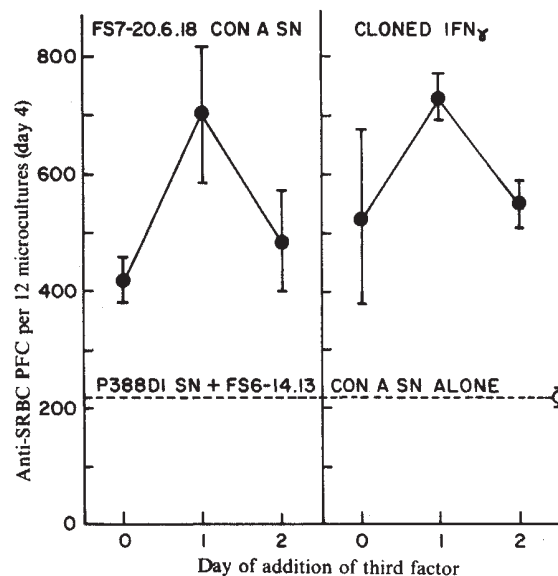


Fig. 2 IFN- γ is a late-acting helper factor. Cultures of purified B cells were set up as described in Fig. 1 legend with optimal concentrations of P388D1 and FS6-14.13 supernatants. Ten units of IFN- γ from either FS7-20.6.18 supernatant or from the cloned gene were added to the cultures on different days. After 4 days' total incubation the cultures were assayed for direct PFC against SRBC. Results shown are the total anti-SRBC PFC found in 12 identical microcultures.

maximum stimulation was achieved with approximately one unit of IFN- γ per culture.

One of the properties distinguishing IL-X from IL-1 and IL-2 is that it can be added considerably later in the B-cell response. Whereas, to be fully effective, IL-1 and IL-2 must be added at the initiation of the response, the addition of IL-X can be delayed 1 or 2 days in a 4-day response¹⁰. To assess whether cloned IFN- γ behaved similarly, we again tested FS7-20.6.18 supernatant and cloned IFN- γ side by side in a kinetic experiment. B-cell cultures received SRBC, IL-1 and IL-2 on day 0 of a 4-day culture. On day 0, 1 or 2 the cultures received FS7-20.6.18 supernatant containing 10 U of IFN- γ or 10 U of cloned IFN- γ . Plaque responses were assayed on day 4. Stimulation of the response was seen again with both interferon preparations and as predicted, both preparations were active even when their addition was delayed 1–2 days, with day 1 being the optimal time of addition (Fig. 2). These results agree with our previous results using this activity isolated from normal T cells and distinguish the IFN- γ activity from that of IL-1 and IL-2.

Interferons have generally been reported as inhibitors of immune responses due to their anti-proliferative effects. However, there are several reports that interferon can be stimulatory, especially when added well into the proliferative phase of the response. In these studies, as in our own previous reports, unpurified interferon was used. The possibility that an associated non-interferon activity was responsible for the immunostimulation has always been a problem, especially when dealing with IFN- γ , which is produced from T cells and is therefore always contaminated with other lymphokine activities. In the present study, the use of IFN- γ produced from a recombinant DNA clone suggested that IFN- γ itself is stimulatory for B-cell antibody production.

In measuring B-cell helper factors *in vitro*, one must always consider the possibility that a particular factor may work indirectly by stimulating non-B cells contaminating the cultures to produce additional activities. We have attempted to minimize this difficulty by developing and thoroughly testing B-cell purification techniques⁹⁻¹¹, and therefore believe it is highly likely that the IFN- γ activity in our experiments is due to its direct effect on the SRBC-responding B cell. This conclusion is

strengthened by the fact that despite an extensive search among normal T cells, hybridoma T cells and macrophage products, we have not identified an activity which can substitute for IFN- γ in this anti-SRBC B-cell response.

It is possible that some contaminant in the IFN- γ preparation from *E. coli*, for example lipopolysaccharide (LPS), is responsible for the effects on B-cell responses described here. The IFN- γ preparation we used is not mitogenic for B cells at concentrations containing 100 U ml⁻¹ IFN- γ . Because effects on our cultures were seen at about 10 U ml⁻¹ IFN- γ and because we have not seen similar effects with LPS at levels below 1 μ g ml⁻¹, we consider this possibility unlikely. Moreover, the activity of our two IFN- γ -containing preparations (FS7-20.6.18 and cloned IFN- γ) is comparable in terms of units of IFN- γ . It is therefore highly unlikely that our results can be accounted for in any way other than that IFN- γ is responsible for the effects we measured.

Many laboratories have described T-cell-derived helper factors which stimulate B-cell antibody production. Such factors, which act late in the B-cell responses and do not seem to stimulate proliferation, have often been called T-cell replacing factor (or TRF) (reviewed in refs 15, 16). These activities have usually been defined in *in vitro* responses of murine B cells and are generally thought of as factors which stimulate the secretion of antibody from proliferating B cells. The question arises as to

whether IFN- γ accounts for all T-cell-derived activities of this sort or whether other lymphokines can also provide this signal to B cells. As activities of this type have been reported in supernatants containing no detectable IFN- γ this is a distinct possibility. On the other hand, we have found that most of the late-acting TRF-like activity in the supernatants of mouse spleen cells stimulated with Con A, which presumably contain many if not all T-cell-derived lymphokines, has the properties of murine IFN- γ , that is, it has an apparent molecular weight of 40,000 on Sephadex gel filtration and is inactivated by incubation at pH 2. These findings suggest that IFN- γ is in fact a major component of TRF activities from normal lymphocytes. The lymphokine(s) responsible for driving the later events of B-cell maturation *in vivo* remains to be identified and will be the subject of future investigations.

Finally, one of the well known effects of IFN- γ which may be significant here is its inhibitory effects on cell proliferation. As IFN- γ is late acting, and in its capacity as a TRF is thought to stimulate differentiation of B cells to antibody secretion, this may be yet another example of a substance which inhibits cell division and simultaneously stimulates differentiation¹⁴.

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1. Sonnenfeld, G., Mandel, A. D. & Merigan, T. C. *Cell. Immun.* **140**, 285-293 (1978).
2. Gresser, I., DeMaeyer-Guignard, J., Torey, M. G. & DeMaeyer, E. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5308-5312 (1979).
3. Nakamura, M., Manser, T., Pearson, G. D. N., Daley, M. J. & Geffer, M. L. *Nature* **307**, 381-382 (1984).
4. King, D. P. & Jones, P. P. *J. Immun.* **131**, 315-318 (1983).
5. Wong, G. H. W., Clark-Lewis, I., McKinn-Breschkin, J. L., Harris, A. W. & Schrader, J. W. *J. Immun.* **131**, 788-793 (1983).
6. Pace, J. L., Russell, S. W., Torres, B. A., Johnson, H. M. & Gray, P. W. *J. Immun.* **130**, 2011-2013 (1983).

7. Zlotnik, A. *et al. J. Immun.* **131**, 794-800 (1983).
8. Steeg, P. S., Moore, R. N., Johnson, H. M. & Oppenheim, J. J. *J. exp. Med.* **156**, 1780-1793 (1982).
9. Leibson, H. J., Marrack, P. & Kappler, J. J. *exp. Med.* **154**, 1681-1693 (1981).
10. Leibson, H. J., Marrack, P. & Kappler, J. J. *Immun.* **129**, 1398-1402 (1982).
11. Harwell, L., Skidmore, B., Marrack, P. & Kappler, J. J. *exp. Med.* **152**, 893-904 (1980).
12. Grey, P. W. *et al. Nature* **295**, 503-508 (1982).
13. Grey, P. W. & Goeddel, D. V. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
14. Okazaki, K. & Holtzer, H. *Proc. natn. Acad. Sci. U.S.A.* **56**, 1484-1490 (1966).
15. Möller, G. (ed.) *Transplant. Rev.* **23** (1975).
16. Möller, G. (ed.) *Immun. Rev.* **63** (1982).

γ -Interferon is one of several direct B cell-maturing lymphokines

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Two classes of molecules often released after the interaction of T lymphocytes, macrophages and antigen are B-cell maturation factors (BMF)¹⁻³ and immune (γ) interferon (IFN- γ)⁴⁻⁷. BMFs directly induce the maturation of resting B lymphocytes to the state of active immunoglobulin secretion¹⁻³, while IFN- γ is defined by the reduction of viral infectivity *in vitro*^{8,9}. However, interferons have been shown to have a variety of effects¹⁰⁻¹⁷ and they have also been reported both to increase and decrease B-cell differentiation in intact animals and complex cellular mixtures *in vitro*¹⁸⁻²⁵. Here we show that murine IFN- γ produced by recombinant DNA technology²⁶ shows similar biological effects to BMFs from two other sources. All three preparations induce immunoglobulin secretion by both normal resting murine splenic B cells and the comparable B-cell tumour line WEHI-279.1 (refs 1, 3). IFN- γ and the other two BMFs are not identical, however, as anti-IFN- γ antibodies block the effects on B cells of IFN- γ , but not those of the other two lymphokines. IFN- γ may be one of several molecules with a direct role in driving the maturation of resting B cells to active immunoglobulin secretion.

We and others have recently begun to use inducible monoclonal cell lines, including tumours, as assay systems for lymphokines^{1-3,27-29} as they overcome problems of interacting cell populations inherent in the use of more complex assay systems. Culture supernatants from the antigen-stimulated monoclonal

helper T-cell line S26.5 (kindly provided by Dr M. Schreier)^{1,30,31} are able to induce terminal differentiation to active IgM secretion in the B-cell tumour line WEHI-279.1 and also in comparable normal resting murine splenic B cells^{1,3}. A second source of such maturational lymphokines is supernatant from cultures of spleen cells from mice homozygous for an autosomal recessive mutation known as viable motheaten (*me^v*). Viable motheaten homozygotes (*me^v/me^v*) show polyclonal immunoglobulin production (mostly of the IgM class) and autoimmune disease³². B-cell maturation in response to these lymphokines does not require interactions with other cells, occurs in most of the B cells present (under optimum culture conditions), is quite rapid (peak immunoglobulin secretion at days 2-3 of stimulation) and involves all of the pre- and post-translational alterations in IgM metabolism known to occur in the transition of normal resting B cells to immunoglobulin-secreting plasma cells^{1,3}. The resulting immunoglobulin secretion has been measured by biosynthetic radiolabelling followed by immunoprecipitation, by enzyme-linked immunosorbent assays or, as done here, by a reverse plaque assay³³ which reveals the number of immunoglobulin-secreting cells in culture at the time of assay. To test directly the effects of IFN- γ in these B-cell maturation assays, we have used IFN- γ produced by CHO cells transfected³⁴ with a plasmid containing the cloned mouse IFN- γ gene²⁶. This IFN- γ should be glycosylated and secreted similarly to that from normal T cells.

Culture supernatants from S26.5 T cells, *me^v* spleen cells and IFN- γ were titrated individually into cultures of either resting spleen cells from normal mice or WEHI-279.1 tumour B cells. The numbers of (immunoglobulin-secreting) plaque-forming cells (PFC) were determined 3 days later by the polyclonal reverse plaque assay³³. As seen in Fig. 1, both normal (a) and tumour (b) B cells responded similarly to all three supernatants with an approximately 10-fold increase in PFC per culture. Cloned IFN- γ is clearly an effective BMF as measured in this

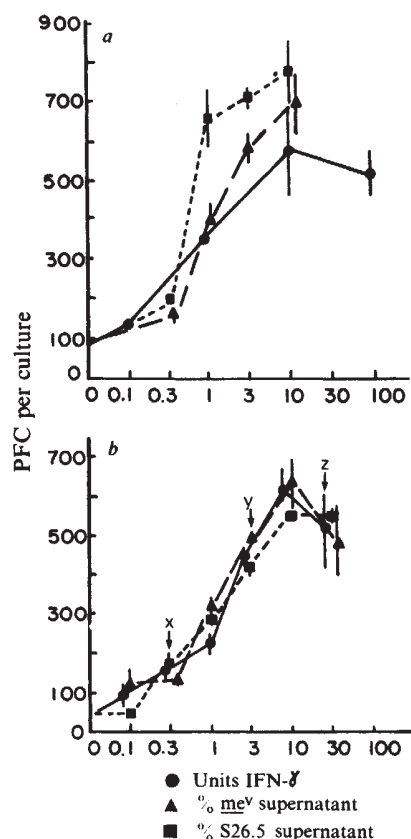


Fig. 1 Dose-response titrations of lymphokines from three sources. Antigen-stimulated S26.5 helper T-cell supernatant (■) (prepared as described¹), non-stimulated *me*^v spleen cell supernatant (▲) (prepared by culturing *me*^v spleen cells at 10^7 per ml for 2 days) and supernatant from CHO cells transfected with plasmid containing the cloned murine IFN-γ gene (●) were tested on a, Percoll gradient-purified (that is, resting) C57BL/6J, DBA/2J or BALB/cBy spleen cells¹, or b, WEHI-279.1 (ref. 37) tumour B cells. Spleen cells were cultured in Iscove's modified Dulbecco's medium supplemented with albumin, transferrin and soybean lipids (IMDM-ATL)³⁸, while WEHI-279.1 cells were cultured in RPMI 1640-based medium containing 10% fetal bovine serum plus other supplements³. Cells were cultured at 10^3 per 0.2 ml flat-bottomed microwell at 37 °C in 5% CO₂/air, and the number of immunoglobulin-secreting cells (PFC) were determined on day 3 by the Protein A reverse plaque assay³³. Percentages of the various supernatants, or amounts of IFN-γ activity were added to the cultures as shown. (The IFN-γ concentration in the undiluted CHO supernatant was 16,000 U ml⁻¹, as tested in an inhibition of cytopathic effect assay using encephalomyocarditis virus as the challenge virus and murine L929 cells as targets⁹). Data shown represent the mean $\pm$ s.e.m. from two to four determinations each. Points x, y and z are referred to in Fig. 2 legend.

direct assay system. Control supernatants derived from CHO cells not transfected with IFN-γ-containing plasmids showed no PFC induction (data not shown).

When tested directly for inhibition of viral infectivity, S26.5 supernatant showed substantial IFN activity (500 U ml⁻¹), while *me*^v supernatant did not show detectable IFN levels (<10 U ml⁻¹). Therefore, we next tested whether anti-IFN-γ antibody (made in rabbits against partially-purified mitogen-induced mouse IFN-γ)³⁵ could inhibit the B-cell responses stimulated by the three different lymphokine preparations (Fig. 2). This antibody has been shown previously to block efficiently the anti-viral action of purified, naturally produced IFN-γ, but not to affect CSF, IL-1 and IL-2 (ref. 36). Varying amounts of IgG from the anti-IFN-γ antiserum were added to cultures containing WEHI-279.1 cells plus either barely stimulatory, almost maximally stimulatory, or supersaturating amounts of the three lymphokine preparations (doses labelled x, y and z respectively, on Fig. 1b). Fig. 2a-c shows the actual PFC responses seen in

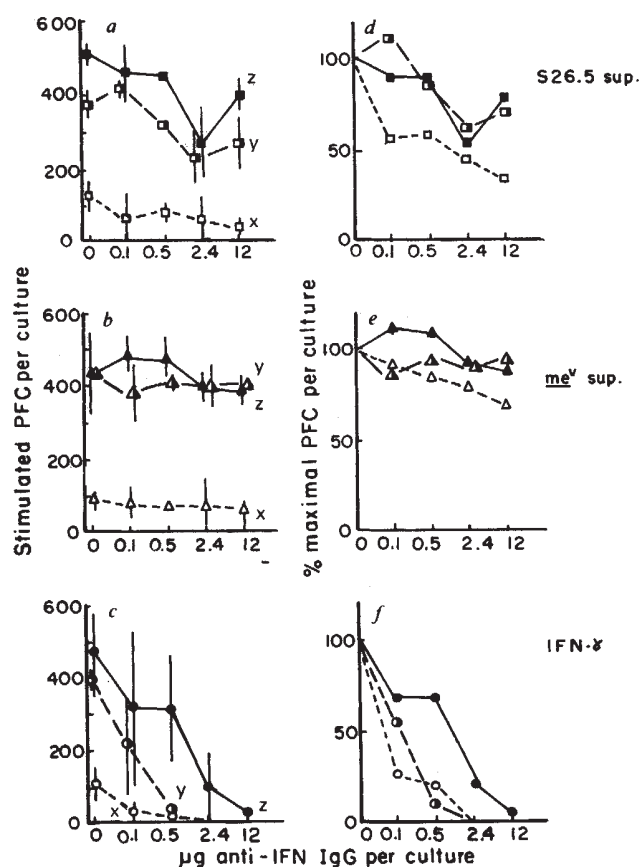


Fig. 2 Inhibition of lymphokine activities by rabbit anti-mouse IFN-γ antibodies. The indicated amounts of anti-IFN-γ IgG (capable of neutralizing 16 units of IFN-γ per μg) were added to the stimulatory lymphokine dose 30 min before WEHI-279.1 cells were added to the cultures. Culture conditions and harvesting were as described in the legend to Fig. 1. Panels a, b and c show stimulated PFC (that is, PFC over background), while panels d, e and f represent the same data expressed as the percentage of the response to that lymphokine concentration but without anti-IFN-γ IgG. a, d, Responses to S26.5 supernatant. b, e, Responses to *me*^v supernatant. c, f, Responses to IFN-γ supernatant. The curves indicated by open symbols and dotted lines refer to the minimum lymphokine concentrations indicated by x in Fig. 1b; the curves indicated by half-closed symbols and dashed lines refer to the near-maximal lymphokine concentrations indicated as y in Fig. 1b; and the curves indicated by closed symbols and solid lines refer to the super-optimal lymphokine concentrations indicated as z in Fig. 1b.

these cultures, while Fig. 2d-f shows the responses as percentages of the maximal response seen with that concentration of lymphokine but without anti-IFN-γ antibody. All doses of IFN-γ were completely inhibited by anti-IFN-γ antibody (Fig. 2c and f), with proportional amounts of anti-IFN-γ being required to inhibit different doses of IFN-γ. At the opposite extreme, no amount of anti-IFN-γ antibody tested produced a significant inhibition of the PFC response to any dose of lymphokine from *me*^v mice (Fig. 2b and e). The responses to supernatant from S26.5 T helper cells were partially inhibited, but no amount of anti-IFN-γ antibody used in these experiments was able to block completely the PFC response to even the lowest amount of S26.5 supernatant (Fig. 2a and d). Normal rabbit IgG was tested as a control for the anti-IFN-γ IgG and showed no inhibitory or stimulatory effects (data not shown). Rabbit (and murine) monoclonal antibodies to recombinant DNA-produced IFN-γ have also recently been tested, and shown to act like the rabbit antibodies to naturally-produced IFN-γ used here (data not shown).

To quantitate how much of the B-cell maturational activity in each lymphokine preparation might be due to IFN-γ, dose-response titrations were performed in the presence and absence

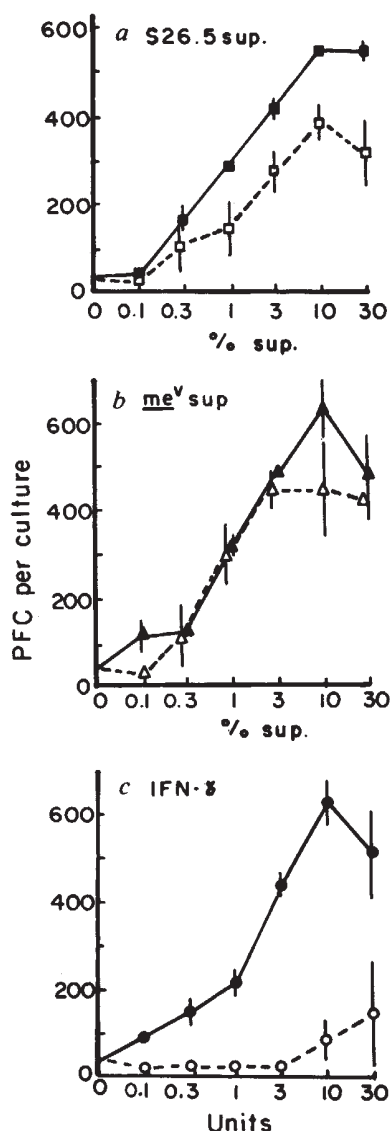


Fig. 3 Comparison of lymphokine titrations in the presence and absence of anti-IFN- γ antibody. The three lymphokine preparations were titrated into cultures of WEHI-279.1 cells as indicated in the legend to Fig. 1b. The solid symbols and lines indicate cultures with no anti-IFN- γ antibody, while the open symbols and dotted lines indicate cultures where 2.4 μ g of anti-IFN- γ IgG (see Fig. 2—enough to block even the highest IFN- γ dose used here) were added to the lymphokines 30 min before the cells. *a*, S26.5 supernatant; *b*, me^v supernatant; and *c*, IFN- γ .

of sufficient anti-IFN- γ antibody to block a 10-fold superoptimal dose of IFN- γ (Fig. 3). As shown in Fig. 3c, the presence of this amount of anti-IFN- γ antibody shifted the dose-response curve for IFN- γ by about 100-fold, indicating that close to 100% of the activity in this supernatant is due to IFN- γ . The dose-response curve of the me^v supernatant (Fig. 3b) was not affected by the anti-IFN- γ antibody, suggesting that little (less than a few per cent) of the activity of this supernatant is due to IFN- γ . About half of the direct B-cell maturational activity in S26.5 supernatant appears to be due to IFN- γ , as this dose-response curve was shifted 2- to 3-fold by anti-IFN- γ (Fig. 3a).

From these experiments it can be concluded that IFN- γ functions as a direct B cell-maturing lymphokine, driving non-immunoglobulin-secreting normal and tumour B cells to active immunoglobulin secretion. In a representative experiment, 200 fluorescent-activated cell sorter (FACS)-purified normal resting BALB/cBy splenic B cells yielded 3 ± 1 PFC per culture on day 3 without added lymphokine, and 130 ± 14 , 150 ± 14 and 130 ± 14 PFC with S26.5, me^v and IFN- γ supernatants, respectively. The

comparable behaviour of monoclonal tumour B cells, normal resting splenocytes and FACS-purified resting B cells suggests that the induction of active immunoglobulin secretion occurs via the direct interaction of IFN- γ and the target B cells, without the required involvement of other cell types or signals. The effects of IFN- γ produced by recombinant DNA technology indicate that the active molecule is indeed IFN- γ , rather than another lymphokine which may also be found in naturally produced IFN- γ preparations. Previous studies have suggested both stimulatory and inhibitory effects of interferons on B-cell function, but always in the context of modulating the effect of another primary inductive signal (either antigen or mitogen) in complex cellular mixtures^{10,18}.

A second conclusion is that at least one, and probably two, other direct B cell-maturing lymphokines exist which are distinct from IFN- γ . These lymphokines may be related to IFN- γ , but do not show the anti-viral effects, nor the major antigenic determinant(s) seen by anti-IFN- γ antibodies, nor identical biochemical properties to IFN- γ . The non-IFN- γ BMF from S26.5 supernatant is less hydrophobic than IFN- γ —fractions 80–104 of the phenyl-Sepharose hydrophobic column shown in Fig. 3 of ref. 2 do not show anti-viral activity, and their BMF activity is not affected by anti-IFN- γ antibodies, whereas fractions 105–130 do show anti-viral activity and their BMF activity is completely blocked by anti-IFN- γ antibodies (data not shown). The me^v lymphokine is both smaller on gel filtration (molecular weight 15,000 compared with 50,000) and more acidic (pI 4 compared with 5.5) than IFN- γ (unpublished results). IFN- γ and the non-IFN- γ BMF molecules from S26.5 and me^v supernatants thus seem to be distinct and separable species.

Since IFN- γ is active in inducing the terminal maturation process which is central to the humoral immune response, and also influences the expression of specific macromolecules and cellular activities in other cell types^{10–25}, it is not clear whether the anti-viral activity for which IFN- γ was named represents its primary physiological function. In any case, the actual *in vivo* role of IFN- γ in humoral immune response induction, and its structural and functional relationships to the other molecule(s) with similar activity, will be important questions for future investigations.

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- Paige, C. J., Schreier, M. H. & Sidman, C. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4756–4760 (1982).
- Sidman, C. L., Paige, C. J. & Schreier, M. H. *J. Immun.* **132**, 209–222 (1984).
- Sidman, C. L. & Marshall, J. D. *J. Immun.* **132**, 845–850 (1984).
- Wheelock, E. F. *Science* **149**, 310–311 (1965).
- Green, J. A., Cooperbrand, S. R. & Kibrick, S. *Science* **164**, 1415–1416 (1969).
- Marcucci, F., Waller, M., Kirchner, H. & Krammer, P. *Nature* **291**, 79–81 (1981).
- Nabel, G., Greenberger, J. S., Sakakeeny, M. A. & Cantor, H. C. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1157–1161 (1981).
- Blalock, J. E. & Gifford, G. E. *J. gen. Virol.* **29**, 315–324 (1975).
- Stewart, W. E. II. *The Interferon System* (Springer, 1979).
- Zlotnik, A. et al. *J. Immun.* **131**, 794–800 (1983).
- Schreiber, R. D., Pace, J. L., Russell, S. W., Altman, A. & Katz, D. H. *J. Immun.* **131**, 826–832 (1983).
- Gidlund, M., Orn, A., Wigzell, H., Senik, A. & Gresser, I. *Nature* **273**, 759–761 (1978).
- Gresser, I., Brouty-Boye, D., Thomas, M. T. & Macieria-Coelho, A. *Proc. natn. Acad. Sci. U.S.A.* **66**, 1052–1058 (1970).
- Crane, J. L., Jr, Glasgow, L. A., Kern, E. R. & Younger, J. S. *J. natn. Cancer Inst.* **61**, 871–874 (1978).
- Wallach, D., Fellous, M. & Revel, M. *Nature* **299**, 833–836 (1982).
- King, D. P. & Jones, P. P. *J. Immun.* **131**, 315–318 (1983).
- Wong, G. H. W., Clark-Lewis, I., McKimm-Breschkin, J. L., Harris, A. W. & Schrader, J. W. *J. Immun.* **131**, 788–793 (1983).
- Braun, W. & Levy, H. B. *Proc. Soc. exp. Biol. Med.* **141**, 769–777 (1972).
- Gisler, R. H., Lindahl, P. & Gresser, I. *J. Immun.* **113**, 438–444 (1974).
- Brodeur, B. R. & Merigan, T. C. *J. Immun.* **113**, 1319–1325 (1974).
- Johnson, H. M., Smith, B. G. & Baron, S. *J. Immun.* **114**, 403–409 (1975).
- Strannegard, O., Larsson, I., Lundgren, E., Miorner, H. & Persson, H. *Infect. Immunity* **20**, 334–339 (1978).
- Vignaux, F., Gresser, I. & Fridman, W. H. *Eur. J. Immun.* **10**, 767–772 (1980).
- Harfast, B., Huddleston, J. R., Casali, P., Merigan, T. C. & Oldstone, M. B. A. *J. Immun.* **127**, 2146–2150 (1981).
- Rodriguez, M. A., Prinz, W. A., Sibbitt, W. L., Bankhurst, A. D. & Williams, R. C. Jr *J. Immun.* **130**, 1215–1219 (1983).
- Gray, P. W. & Goeddel, D. V. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5842–5846 (1983).
- Gillis, S., Fern, M. M., Ou, W. & Smith, K. A. *J. Immun.* **120**, 2027–2032 (1978).
- Muraguchi, A. et al. *J. Immun.* **127**, 412–416 (1981).

29. Pure, E. *et al.* *J. Immun.* 127, 1953–1958 (1981).
30. Schreier, M. H. & Tees, R. *Int. Archs Allergy appl. Immun.* 61, 227–237 (1980).
31. Schreier, M. H., Tees, R. & Nordin, A. A. *Lymphokines* 5, 443–464 (1982).
32. Shultz, L. D., Coman, D. R., Bailey, C. L., Beamer, W. G. & Sidman, C. L. *Am. J. Path.* (in the press).
33. Gronowicz, E., Coutinho, A. & Melchers, F. *Eur. J. Immun.* 6, 588–590 (1976).
34. Gray, P. W. *et al.* *Nature* 295, 503–508 (1982).
35. Osborne, L. D., Georgiades, J. A. & Johnson, H. M. *Immunology* 53, 65–70 (1980).
36. Farrar, W. L., Johnson, H. M. & Farrar, J. J. *J. Immun.* 126, 1120–1125 (1981).
37. Sibley, C. H. *et al.* *J. Immun.* 125, 2097–2105 (1981).
38. Schreier, M. H. & Tees, R. in *Immunological Methods* Vol. II (eds Lefkowitz, I. & Pernis, B.) 263–275 (Academic, London, 1981).

Cellular transformation by coordinated action of three peptide growth factors from human platelets

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Platelet-derived growth factor^{1–4} (PDGF) is known to be involved in regulating the mitosis of connective tissue cells, and recent studies have also shown that it may function in mediating cellular transformation^{5–8}. The oncogene carried by simian sarcoma virus, *sis*, is homologous to one chain of PDGF^{5–8}, and treatment of non-neoplastic cells with this growth factor results in increased transcription of another oncogene, *myc* (ref. 9). PDGF also stimulates the synthesis of proteins that are characteristic of transformed cells¹⁰. However, phenotypic transformation does not appear to result from the action of PDGF alone. For example, expression of *myc* does not transform cells in the absence of other oncogene expression¹¹. We have recently shown that platelets contain another peptide growth factor, transforming growth factor- β (TGF- β)^{12,13}, in addition to PDGF. We report here that extracts of human platelets can induce anchorage-independent growth of non-neoplastic rat kidney (NRK) fibroblasts, but that purified PDGF alone does not elicit this effect. Rather, the transforming activity of the platelet extract is due to a concerted action of three distinct peptides: PDGF, TGF- β and a newly identified analogue of epidermal growth factor (EGF).

NRK fibroblasts are non-neoplastic, contact-inhibited cells that are indicators of phenotypic transformation. With the appropriate stimulus, these cells undergo anchorage-independent growth as assayed by colony formation in medium containing agar^{14,15}. An acid-ethanol extract of human platelets induces phenotypic transformation of NRK fibroblasts (Fig. 1a). Homogeneous PDGF¹⁶ does not induce anchorage-independent growth of the cells even when used at concentrations 10–20-fold greater than that required for half-maximal stimulation of DNA synthesis (Fig. 1b).

The results shown in Fig. 1 suggest that other components in the platelet extract, acting alone or in combination with PDGF, are responsible for the induction of phenotypic transformation. In fact, previous studies have shown that platelets contain a type- β transforming growth factor^{12,13}. Figure 2a shows the elution position of this peptide during gel-filtration on a column of Bio-Gel P-60. TGF- β can induce anchorage-independent growth of NRK cells, but expression of this biological activity requires a synergistic interaction with EGF or a peptide resembling EGF (TGF- α)^{12,17,18}. We can detect a new EGF-like peptide in human platelets by gel-filtration of the extract, adding purified TGF- β ¹² to aliquots of the column fractions, and then assaying the samples for the ability to induce anchorage-independent growth (Fig. 2b). Column fractions 80–90 contain EGF-like activity as determined by synergism with TGF- β in the bioassay (Fig. 2b) and competition with ¹²⁵I-labelled EGF in a radioreceptor assay (Fig. 2c). Control studies showed that the biological activity of these fractions decreased at least 10-fold when assayed in the absence of TGF- β . The apparent molecular weight (M_r) of this platelet-derived, EGF-like peptide is 27,000

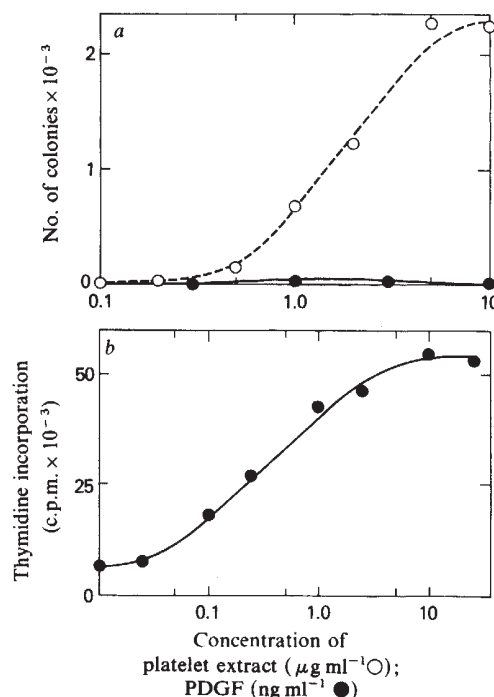


Fig. 1 Relative transforming activity of platelet extracts and PDGF. The acid-ethanol extract of human platelets was prepared and quantitated as described elsewhere¹². a, The ability of the extract (○) or homogeneous PDGF¹⁶ (●) to induce anchorage-independent growth of NRK fibroblasts as measured by the formation of colonies (>3,000 μm^2 per dish, ordinate) in soft agar. The concentrations of extract and PDGF (abscissa) are shown on logarithmic scale. This assay was performed in medium containing calf serum (10%, Gibco)^{12,41}. b, The ability of purified PDGF to stimulate ³H-thymidine incorporation into NRK fibroblasts (see ref. 12 for procedures).

as determined by gel-filtration (Fig. 2) and 24,000 as determined by preparative SDS-polyacrylamide gel electrophoresis¹⁹. (Fractions 84–88 were pooled, freeze-dried, and an aliquot of the residue was run on a 12.5% SDS gel. The gel was sliced, and eluted proteins²⁰ were assayed for the ability to induce anchorage-independent growth of NRK fibroblasts in the presence of TGF- β .) The EGF-like biological activity of this polypeptide shows that it is not the binding protein for EGF^{21,22}. Its stability to both acidic pH (see Fig. 2 legend) and SDS-gel electrophoresis shows that it is distinct from the EGF-binding protein complex^{21,22}. It was also possible that the 'new' peptide was PDGF, because PDGF, acting through its own receptor, can inhibit the binding of labelled EGF to the EGF receptor^{23,24}. The EGF-like peptide identified in Fig. 2 is not PDGF; this new peptide competes with ¹²⁵I-labelled EGF in a radioreceptor assay using cells, line A431, that lack PDGF receptors^{25–27} (Fig. 2c).

Oka and Orth²⁸ have recently suggested that human platelets contain both pro- and authentic EGF (M_r 33,000 and 6,000) as determined by reactivity in a human EGF immunoassay. The relationship between the larger peptide they detect and the EGF-like activity we detect using receptor binding competition (see Fig. 2) is not clear, but specific chemical modification studies indicate that the new growth factor described in Fig. 2 is intrinsically distinct from EGF (Fig. 3). Treatment of this platelet peptide with cyanogen bromide (CNBr) does not alter its affinity for the EGF receptor (Fig. 3b) despite the fact that: (1) amino acid analysis of the partially purified platelet peptide before and after CNBr treatment showed that cleavage at methionyl residues was complete, (2) EGF treated in parallel with CNBr showed the expected decrease^{29,30} in receptor binding affinity (Fig. 3a), and (3) deliberate contamination of pure EGF with a large excess of protein (so that it was less pure than the preparation of the platelet-derived EGF-like peptide;

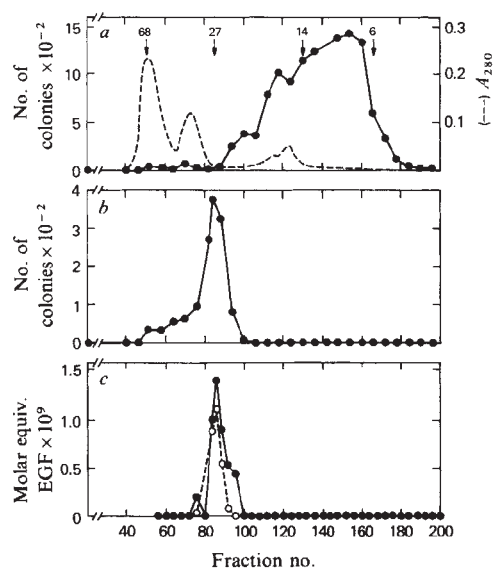


Fig. 2 Gel filtration of the human platelet extract on Bio-Gel P-60. *a*, Protein profile as determined by absorbance at 280 nm (---). Vertical arrows show, from left to right, the elution positions of bovine serum albumin ($M_r = 68,000$; V_0), chymotrypsinogen A ($M_r = 27,000$), lysozyme ($M_r = 14,000$) and insulin ($M_r = 6,000$), respectively. ●, Elution position of platelet-derived TGF- β determined by colony formation ($>9,000 \mu\text{m}^2$) induced in the presence of pure murine EGF⁴² (0.5 nM) and 2.5 μl of selected column fractions. *b*, Elution position of the platelet-derived, EGF-like peptide determined by colony formation ($>9,000 \mu\text{m}^2$) induced in the presence of homogeneous TGF- β (20 pM, prepared as described in ref. 12) and 250 μl of selected column fractions. *c*, Elution position of material that competes for the binding of ¹²⁵I-labelled EGF to the EGF receptor of NRK fibroblasts (●) and A431 cells (○).

Methods: The acid-ethanol extract of platelets (250 units) was solubilized in 1 M acetic acid (100 ml) and applied to a column (10 $\times$ 100 cm) of Bio-Gel P-60 which had been equilibrated in the sample solvent (1 M acetic acid, pH ~ 2). Fractions containing 35 ml were collected at a flow rate of 100 ml h⁻¹. The radioreceptor assay was performed similarly to the described procedure³⁴. Cells were suspended (2×10^5 NRK cells ml⁻¹ or 1×10^4 A431 cells ml⁻¹) in medium (Dulbecco's modified Eagle's medium with gentamycin) containing 10% fetal bovine serum (Bio-Fluids, Rockville, Maryland), seeded in 24-well plates (1 ml of the cell suspension per well), and incubated overnight (37 °C, humidified atmosphere of 10% CO₂ in air). Aliquots of selected column fractions (120 μl) were dried in the presence of bovine serum albumin (20 μg) in a vacuum desiccator. The residues were dissolved in 4 mM HCl (20 μl), diluted with binding buffer³⁴ (0.2 ml) containing ¹²⁵I-labelled EGF (about 2×10^5 c.p.m.), and incubated with washed cells as described previously³⁴. Receptor binding competition data were converted to molar equivalents of EGF using pure murine EGF as the standard.

see Fig. 3 legend) did not prevent its reaction with CNBr. Thus, the new EGF analogue in human platelets lacks a methionine residue critical to receptor binding.

Figure 4 shows the biological interactions between the three platelet-derived growth factors described above. In this experiment, cells were cultured in soft agar containing plasma-derived serum (rather than whole-blood derived serum) in order to decrease the basal levels of platelet peptides. Homogeneous PDGF in combination with either TGF- β alone or murine EGF alone does not efficiently induce phenotypic transformation (Fig. 4a). Similarly, TGF- β in conjunction with murine EGF can only minimally stimulate anchorage-independent growth in this system if the concentration of added PDGF is less than 0.1 ng ml⁻¹ (Fig. 4a). When platelet-derived peptides are absent from the basal assay medium, phenotypic transformation requires the addition of all three growth factors (Fig. 4a). In three separate experiments, PDGF (3 ng ml⁻¹) 'restored' the

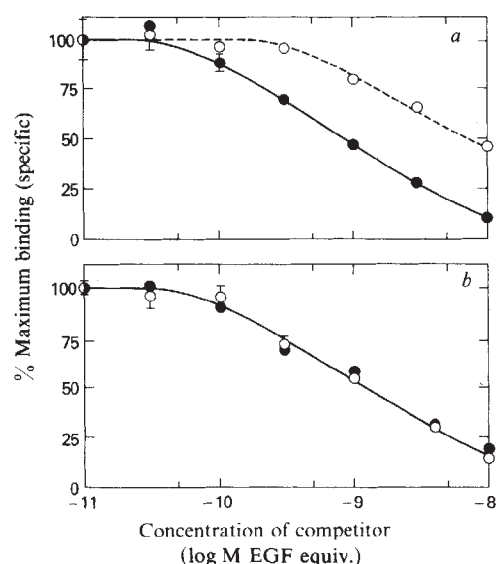


Fig. 3 The effect of CNBr on the receptor binding affinity of murine EGF (*a*) and the human platelet-derived EGF analogue (*b*). Results were obtained by measuring the ability of these peptides to compete with ¹²⁵I-labelled EGF for binding to the EGF receptor of NRK fibroblasts. The binding of labelled EGF to the cells was performed as described in Fig. 2 legend. Samples contained selected amounts of EGF or the platelet-derived EGF analogue, native (●) or after treatment with CNBr (○). Maximal binding of labelled EGF (determined in the absence of unlabelled competitor) was about 1,000 c.p.m.; nonspecific binding (determined in the presence of 10^{-7} M EGF) was about 150 c.p.m.

Methods: Treatment of EGF (0.5 nmol, 1 residue methionine per mol EGF^{35,36,42,43}) and the partially purified EGF analogue (4×10^{-12} mol equivalents of EGF as determined by receptor binding competition; 0.5 nmol methionine in total protein as determined by amino acid analysis) with CNBr (0.25 μmol per sample) was performed in 70% formic acid (50 μl per sample) for 20 h at 23 °C. Native samples of EGF and the EGF analogue were treated in parallel in the absence of CNBr. After the reaction period, portions (10%) of the samples were removed for amino acid analysis. (Methionine values were decreased $>90\%$ by the CNBr treatment; other amino acids were not affected.) To ensure complete removal of CNBr and formic acid, the remainder of each sample was dried under a gentle stream of nitrogen (in the presence of bovine serum albumin, 100 μg), the residues were dissolved in 4 mM HCl (200 μl), and the resulting solutions were freeze-dried overnight. For the control studies, EGF (70 pmol) was deliberately contaminated with a large excess of protein (myoglobin, 60 nmol) before treatment with CNBr. The data are plotted as the mean of duplicate determinations using error bars to show the range (when observed).

activity of TGF- β (20 pM) and murine EGF (0.5 nM) to 50, 100 and 65% of that observed with autologous 10% whole-blood derived serum. Furthermore, Fig. 4b shows that in a plasma-derived system, the human platelet-derived EGF analogue efficiently synergizes with PDGF and TGF- β to induce transformation.

Recent studies have emphasized the relationships between PDGF and oncogenesis⁵⁻¹⁰. The results described here show that PDGF alone does not induce transformation in NRK cells, but that it acts in concert with platelet-derived TGF- β and EGF-like peptides. These data agree well with previous studies indicating that PDGF controls proliferation by modulating the response of cells to other growth factors³¹⁻³³. Mechanistically, TGF- β can increase the number of cell-surface receptors for EGF and alter their down-regulation in response to ligand binding³⁴. The platelet-derived EGF analogue can bind to these receptors (Figs 2, 3) and exert EGF-like effects (Fig. 4). This

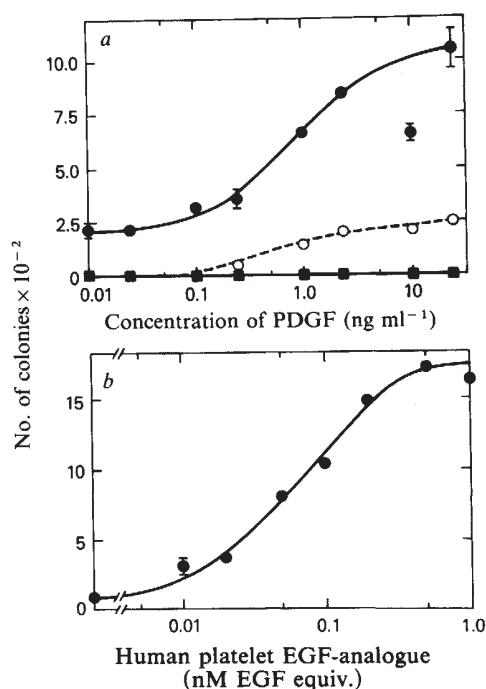


Fig. 4 Biological interaction of the three platelet-derived growth factors. The amount of PDGF in the samples was varied (abscissa, logarithmic scale). The concentrations of TGF- β and EGF were constant at 20 pM and 0.5 nM, respectively. *a*, Colony-forming activity of NRK fibroblasts (colonies $>3,000 \mu\text{m}^2$) in the presence of PDGF, TGF- β and EGF (●); PDGF and TGF- β (■); or PDGF and EGF (○). *b*, The ability of the human platelet-derived EGF analogue to induce colony formation of NRK cells in soft agar when assayed in medium containing 10% plasma-derived serum, TGF- β (20 pM) and PDGF (3 ng ml⁻¹).

Methods: The assay was performed as before^{12,41} except that the medium contained 10% plasma-derived serum (bovine) rather than 10% calf (whole blood-derived) serum. TGF- β , PDGF and murine EGF were purified to homogeneity^{12,16,42}. The platelet-derived EGF analogue was partially purified by gel filtration (see Fig. 2) and HPLC on a C₁₈ column (Vydac, semipreparative) using 0.1% trifluoroacetic acid (TFA) as the stationary phase and 0.1% TFA in an acetonitrile gradient as the mobile phase (10–40% acetonitrile in 3 h, 0.8 ml min⁻¹). Fractions containing 0.8 ml were collected. The elution position of the EGF analogue (about 15% acetonitrile) and its quantitation were determined by assaying aliquots of column fractions in the radioreceptor assay with A431 cells (see Fig. 2 legend for details). Plasma-derived serum (10% in medium) was prepared by adding fresh bovine plasma (11 ml) to medium (100 ml of Dulbecco's modified Eagle's medium containing gentamycin). A fibrin clot formed and retracted during overnight incubation at 37°C. After removal of the clot, the resulting solution was sterilized by filtration. Autologous bovine whole-blood derived serum (10% in medium) was prepared similarly using the serum from freshly clotted whole blood. The plasma and serum were derived from the same bleeding.

new growth factor is structurally distinct from authentic EGF as judged by its apparent larger molecular weight and by the absence of a methionine residue critical to receptor binding (the methionine at position 21 in both human³⁵ and murine³⁶ EGF). In the latter regard, this analogue resembles the type α transforming growth factors^{15,17} which are EGF-like peptides lacking methionine^{37,38}. Similarly, this critical methionine residue is absent from the potential EGF-related peptides encoded in the mouse EGF mRNA^{39,40}. Our results show that the human platelet contains at least three distinct growth factors and that, in the presence of plasma, the interaction of these three peptides can induce transformation of fibroblastic cells.

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- Ross, R., Glomset, J., Kariya, B. & Harker, L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1207–1210 (1974).
- Kohler, N. & Lipton, A. *Expl. Cell Res.* **87**, 297–301 (1974).
- Antoniades, H. N., Stathakos, D. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2635–2639 (1975).
- Antoniades, H. N. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1973–1977 (1977).
- Waterfield, M. D. *et al. Nature* **304**, 35–39 (1983).
- Doolittle, R. F. *et al. Science* **221**, 275–277 (1983).
- Robbins, K. C., Antoniades, H. N., Devare, S. G., Hunkapiller, M. W. & Aaronson, S. A. *Nature* **305**, 605–608 (1983).
- Deuel, T. F., Huang, J. S., Huang, S. S., Stroobant, P. & Waterfield, M. D. *Science* **221**, 1348–1350 (1983).
- Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. *Cell* **35**, 603–610 (1983).
- Scher, C. D., Dick, R. L., Whipple, A. P. & Locatelli, K. L. *Molec. cell. Biol.* **3**, 70–81 (1983).
- Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304**, 596–602 (1983).
- Assoian, R. K., Komoriya, A., Meyers, C. A., Miller, D. M. & Sporn, M. B. *J. biol. Chem.* **258**, 7155–7160 (1983).
- Childs, C. B., Proper, J. A., Tucker, R. F. & Moses, H. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5312–5316 (1982).
- Kahn, P. & Shin, S. *J. Cell Biol.* **82**, 1–16 (1979).
- De Larco, J. E. & Todaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4001–4005 (1978).
- Antoniades, H. N., Scher, C. D. & Stiles, C. D. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1809–1813 (1979).
- Roberts, A. B., Frolik, C. A., Anzano, M. A. & Sporn, M. B. *Fedn Proc.* **42**, 2621–2626 (1983).
- Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M. & Sporn, M. B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5339–5343 (1981).
- Laemmli, U. K. *Nature* **227**, 680–685 (1970).
- Roberts, A. B. *et al. Biochemistry* **22**, 5692–5698 (1983).
- Taylor, J. M., Cohen, S. & Mitchell, W. M. *Proc. natn. Acad. Sci. U.S.A.* **67**, 164–171 (1970).
- Taylor, J. M., Mitchell, W. M. & Cohen, S. *J. biol. Chem.* **249**, 3198–3203 (1974).
- Bowen-Pope, D. F., DiCorleto, P. E. & Ross, R. *J. Cell Biol.* **96**, 679–683 (1983).
- Collins, M. K. L., Sinnott-Smith, J. W. & Rozengurt, E. *J. biol. Chem.* **258**, 11689–11693 (1983).
- Fabricant, R. N., De Larco, J. E. & Todaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 565–569 (1977).
- Heldin, C.-H., Westermark, B. & Wasteson, A. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3664–3668 (1981).
- Bowen-Pope, D. F. & Ross, R. *J. biol. Chem.* **257**, 5161–5171 (1982).
- Oka, Y. & Orth, D. N. *J. clin. Invest.* **72**, 249–259 (1983).
- Schlechter, Y., Hernaez, L., Schlessinger, J. & Cuatrecasas, P. *Nature* **278**, 835–838 (1979).
- Schreiber, A. B., Yarden, Y. & Schlessinger, J. *Biochem. biophys. Res. Commun.* **101**, 517–523 (1981).
- Stiles, C. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 1279–1283 (1979).
- Clemmons, D. R., Van Wyk, J. J. & Pledger, W. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6644–6648 (1980).
- Wharton, W., Leof, E., Olashaw, N., O'Keefe, E. J. & Pledger, W. J. *Expl. Cell Res.* **147**, 443–448 (1983).
- Assoian, R. K., Frolik, C. A., Roberts, A. B., Miller, D. M. & Sporn, M. B. *Cell* **36**, 35–41 (1984).
- Gregory, H. *Nature* **257**, 325–327 (1975).
- Savage, C. R., Inagami, T. & Cohen, S. *J. biol. Chem.* **247**, 7612–7621 (1972).
- Marquardt, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4684–4688 (1983).
- Massagué, J. *J. biol. Chem.* **258**, 13606–13613 (1983).
- Gray, A., Dull, T. J. & Ullrich, A. *Nature* **303**, 722–725 (1983).
- Scott, J. *et al. Science* **221**, 236–240 (1983).
- Roberts, A. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 3494–3498 (1980).
- Savage, C. R. & Cohen, S. *J. biol. Chem.* **247**, 7609–7611 (1972).
- Cohen, S. & Carpenter, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1317–1321 (1975).

Human epidermal growth factor receptor cDNA is homologous to a variety of RNAs overproduced in A431 carcinoma cells

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The recently discovered similarity between the human epidermal growth factor (EGF) receptor and the avian erythroblastosis virus *v-erb-B* protein¹ supports the hypothesis that viral oncogenes share a common evolutionary origin with genes encoding growth-regulating cell-surface receptors. To elucidate the relationship between receptors and malignant transformation, we have now used a fragment of *v-erb-B* as a probe to screen a cDNA library of mRNA from A431 human carcinoma cells, which possess a large number of EGF receptors². Of the six clones isolated, the largest (pE7)

contains an insert of 2.4 kilobase pairs (kbp) whose deduced amino acid sequence is homologous to the *v-erb-B* protein and identical to reported EGF receptor peptide sequences¹. This pE7 cDNA hybridized to three prominent RNAs of ~10, 5.6 and 2.9 kilobases (kb), and to three minor species of 6.3, 4.6 and 3.3 kb. All were present in elevated levels in A431 cells. The prominent 2.9-kb RNA was homologous only to the 5' portion of the pE7 insert. This result raises the possibility that differential RNA processing is used by A431 cells to generate a variety of RNAs.

EGF elicits a wide variety of rapid and delayed responses mediated via specific high-affinity cell-surface receptors³. These responses to EGF are dependent on the occupation of functional receptors at the cell surface. Short-term effects of EGF include activation of transport systems⁴⁻⁶ and increased protein phosphorylation⁷. Delayed responses include stimulation of DNA, RNA and protein synthesis that ultimately results in cell proliferation⁸⁻¹⁰.

The EGF receptor is a glycoprotein of molecular weight 170,000 which has been identified in many types of cells¹¹⁻¹⁵.

The receptor has an EGF-stimulated protein kinase activity, which phosphorylates itself and other substrates on a tyrosine residue⁷. Regulation of the cellular response to EGF can occur through ligand-induced 'down regulation' of the receptor¹⁶⁻²⁰.

Recently, six different peptides from the EGF receptor have been isolated, sequenced and found to be strikingly similar to the deduced amino acid sequence of v-*erb-B*¹. The latter is the protein product of the *erb-B* gene of avian erythroblastosis virus (AEV) which induces both erythroblastosis and sarcomas in infected birds^{21,22}. Most mammalian cells contain homologous counterparts to the viral transforming genes^{23,24}, whose enhanced expression is thought to produce the malignant phenotype²⁵⁻²⁷. The strong similarity in protein structure between v-*erb-B* and the EGF receptor supports the idea that the human cellular homologue to v-*erb-B* (c-*erb-B*) may be the same gene as that coding for the EGF receptor. This hypothesis is reinforced by the finding that the c-*erb-B* gene and the EGF receptor gene are both localized on the same region of human chromosome 7 (refs 28, 29).

Fig. 1 Nucleotide sequence and deduced translation of the pE7 cDNA insert. The nucleotide sequence of the coding strand of clone pE7 is presented with the 5'-terminal *Cla*I site as position number 1. The amino acid sequence, represented by single-letter abbreviations, is shown directly above the DNA sequence. The translation of the *v-erb-B* (ref. 30) gene product is aligned with the pE7 translation and presented above it, beginning at nucleotide 1,305. Identical predicted amino acid sequences are indicated by asterisks (*). To optimize the similarities between two sequences, a nucleotide triplet has been removed at position 1,476, indicated by (—). Lightly underlined amino acid triplets represent potential *N*-linkage glycosylation sites, heavily underlined sequences indicate the location of the likely transmembrane region of the protein. A dot (•) points to the putative kinase phosphoacceptor^{7,44}. Boxed sequences represent those amino acids shared with published EGF receptor peptide sequences¹. The nucleotide sequence corresponding to the *v-erb-B* *Bam*HI fragment used to screen the library is located at 1 756-2 314.

Methods: Poly(A)⁺ RNA was isolated from total A431 RNA⁴⁵ and used as a template for AMV reverse transcriptase synthesis of cDNA using oligo(dT) as primer^{46,47}. After second strand synthesis⁴⁷, the cDNA was treated with S₁ nuclease (1 U μ l⁻¹), blunt-end ligated to *Cl*aI artificial linkers, restricted with *Cl*aI, and after fractionation over Sepharose 4B to remove cDNA <500 bp, the cDNA was ligated into *Escherichia coli* N38. A 0.5-kbp *B*amHI *v-erb-B* fragment^{30,31} was nick-translated⁴⁸ and used as probe to screen the ~10,000 clone library. One clone (pE7) was sequenced by the Sanger 'dideoxy' method⁴⁹. The 2.4-kb insert was excised from pBR322 by restriction endonuclease cleavage with *Cl*aI and purified by preparative electrophoresis on low melting point 1.0% agarose gels⁵⁰. After further digestion with selected restriction endonucleases, fragments of the cDNA insert were ligated into the appropriate molecular cloning sites of bacteriophage M13 substrains mp-8-9, or -19 (ref. 51). After transfection into *E. coli* strain JM-101, single-stranded recombinant phage DNAs were isolated⁵². All DNA sequences were obtained by the dideoxy nucleotide chain termination method^{49,52}. The single-stranded subclones were selected first at random and later by hybridization to overlapping fragments cloned in bacteriophage M13 in the opposite orientation. All M13 inserts were sequenced using a flanking universal primer⁵³. The complete contiguous sequence was assembled from individual, overlapping subclones containing fragments in either orientation, using the programs described by Staden⁵⁴ modified to run on an IBM-3081 computer.

1 R C A W R R A F S S N N P A L C N V E S I Q W R D I V S S D F L S N M S H D F Q N 120
ATCGATGTGATGCGCGCGTGGCTTCAGCAACAACCGCTGCCCTGTCAACGTGGAGAGCATCAAGTGGCGGACATAGTCAGCAGCTGATCTTTCAGCAACATGTGCGAGCTTCCAGA

121 H L G S K S S V I Q A V P M G A C W G A G E E N C Q K L T K I I C A Q Q C S G R 240
ACCACCTGGCGAGCTGCAAAAGTGTGATCGCAAGCTGTCCAACTGGGAGCTTGTGGGGTGCGAGGAGGAGAACTGCCGAAATCATCTGTGCCAGCAGCTGCTCCGCG

241 S R G K S P S D C H N Q C A A G C T G P R E S D C L V C R F D E A T C K D 360
GCTCGGTGGCAAGTCCCGCAGTCACTGTGCGCAACACGATGTGTCAGAGTGCACAGGCCCCGGGAGAGCACTGCTGCTGTCCGCAAAATTCGAGACGAGCAACCGCAAGG

361 T C P P L M L Y N P T T Y Q M D V N P E G K Y S F G A T C V K K C P R N Y V V T 480
ACACCTGCCCCACTCATGCTCTACAACCGCCACAGTACCAAGTGGATGTGAACCCGAGGGCAAAATACAGCTTTGTGCCACTGCGTGAAGAAGTGTCCCGTAATTATGTGGTGA

481 D H R A C V R A C G A D S Y E M E E D A V R K C K K C E G F C R K V C N C I G I 600
CAGATCACCTGCTGCTGCGAGCTGTGGGGCGACAGCATAGATGAGGAGAACGGGTCGCCAGCTTAAGAGTGCAGAGGGCTGTCCGCAAAAGTGTGTAACGGAAAGTGA

601 G E F G K D S L S I N A T N I K H F K N C T S I S G D L H I L V A F R G D S F T H 720
TTGGTGAATTAAAGACCTCACTCCATAATGTACGAATATAAACCCTCAAAACCTGACCTCATCAAGTGGCATCTCCACACTCTGGTGGCAATTAGGGGTACTGCTTCACG

721 T P P L D P Q E L D I L K T V K E I T C F L L I Q A W P E N R T D L H A F E N L 840
ATACTCTCTCTGATCCACAGGAAGTGGATATTGTGAACCGTAAGGAAGATCACAGGGTTTTGTGTATTCAGGCTGGCGTGAACAGGAGGAGCTCATGCTTTCAGAAC

841 E I I R G R T K Q H G Q F S L A V V S L N I T S L G L R S L K E I S D G D V I I 960
TAGAAATCATACGGCGGAGCAACAGCAACATGCTCAGTTTCTCTTGCAGCTGTCAAGCTGAACAATCAACTCTGGGATACAGCTCTCCAGGAGATAGTGAATGAGATGTGTAA

961 S G N K N L C Y A N T I N W K K L F G T S G Q K T K I I S N R G E N T G A A A C A G C T C A A G G C A C C 1080
TTTCAGSAAACAAAATTTGTCTATGCAATAACATAAACTGGAAAAAAGCTTTTGGACCTGCGGTGAGAAACAAAATATATAAGCAACAGAGGCTGAACACGCTCGCAAGGCTAC

1081 G S A M F C A P R G C W G R A D C V S C R N V S R G R E C V D K C N L L E G E 1200
AGGGGTCTCCACTGCTGTGCTCCCGAGGCTCTGGGGCGAGCGAGGATGTGCTCTTCCGGAATGTACCGGAGCGGGAATGGCTGGACAGTGAACCTCTGAGAGGTG

1201 P R E F V E N S E C I Q C H P E C L P Q A M N I T C T G R G P D N K K K I Q C A H Y 1320
AGCCAGAGGAGTTTGTGGAACTCTGAGTGATACAGTGCACCGCAGAGTGGCTGCTCAGGCGATGAACATCACTGCAAGGACGGGGACGACAGCACTGTATCCAGTGTGCCACT

1321 * * * * * A * * * * * L * * * * * D * * * * * N * * * * * A * * * * * Q * * * * * F * * * * *
I D G P H C V K T C P A G V M G E N N T L V W K Y A D A G N V C H L C R P R C T
ACATTGGCGCGCCCACTGGCTCAAGCAGCTGGCGCGGAGGTTGATGGGAGAAACAAACCACTGGCTTGAAGATACGAGACCGCGGCACTGTGTGCCACTGTGGCATGCCAACTGCA

1441 R * * * * * * * * * * - * * * * T * * * * * A * * * * * C * * * * * G * * * * * G * * * * *
Y G C T G P G L E G C C T N G P K I P S I A T C M V G A L L L L V A L C I G
CTCAGGATGCACTGGCGCAGGTCTGAAGGCTGTCCACGAATGGCGTAAAGTCCCGTCCATCGGCACATGGGATGCTGGGGCCCCCTCTCTTGCTGTGGTGGCTGGGAGATG

1442 * * * * * Y L R * H * * * 1560
L F I C R R H I V K K A T L R R L L Q E R E L V E P L T P S G E A F N Q A L L N
GCCCTCTCATCGAAGGGCCCACTGTTCGGAAGACCACTGCGGGAGGCTGCTCGGAGAGGAGGCTGTGGAGGCTCTACACGCTCAGTGAGAGAGCTGCCACCAAGCTCTCTGA

1561 *
I L K E T E P K K I X [V L G S C A F G T V Y K I G L W I P E G E K] V K I P V A I K
GATCTTGAAGGAACTGAATCAAAAAGATCAAACTGCTGGCGTCGGGTGCGGACGGTGTATAAGGAGCTTGCATCCGACAGAGTGAGAAAGTTAAAACTCCGCTGGCGATCA

1681 *
E L R A G T S P K A N K E I L D A Y W A S V D N P H V C R L L C I C L T S T
AGGAATTAAGAGAGCAACATTCGGAAGAGCAAGAGAAATCTGTGATGAAGCTAGCTGATGCGCGAGGTGGAACAACCCCGACTGTGCCCGCTGTGGCAGTCTGCTCACTCCA

1801 *
V Q L I T A C L M P S A C T L L D T V R E H K D N I G S R Y L L N W C V Q I A K H
CGTGCAGCATCAACGAGCTCATGCTCTGGCTGGCTGCTGACTGATGTCCGGGAAACAAAGACAATATGGCTGCAGTACTGCACTCAAGTGTGTGACAGTCCGAAGAGGCA

1921 * * * * * E *
N Y L E D R R L V H R D L A A R N V L V K T P Q H V K E I T D F G L A K L L G A E
TGACTACTTGAGAGCGCTGCTGTGTGCAACGGCAGCTGGCAGGCGAGCAAGCTATGGTGAAAACACCGACGATGTCAAGATACAGAGTTTGGGTGGGCAAACTGCTGGGTGGG

2041 *
E K E Y H A E G G K V P I K W M A L E S I L H R I Y T H Q S D V W S Y G V T V W
AAGAGAAGATACCATCGAGAGAGGCGAAGTGCCATCAATGAGTGGATGGCATTGAATCAATTTACACAGAACTATATCCACCCAGAGTATGCTGGAGCTACGGGTGACGGTTT

2161 *
A G T M T G A C K P Y D A G T I P A S E T S I L E K G E R L P Q P P I C T I D
GGGACTTGATGACCTTTGGATCGAAGCATCATGAGGAATCCCTGCGACGAGATCTTCCCTCATCTGGAAAGGAGAACGGCTCCCTCAGCCAGGAGATGATGACCATGAT

2281 2393

Here we report the isolation and characterization of cDNA clones obtained from an A431 cDNA library using a *v-erb-B* probe from the 'src family' region that is known to be conserved among oncogenes^{30,31}. The plasmid pEB1 containing the *v-erb-B* sequence was given by Dr Tadashi Yamamoto (University of Tokyo). Double-stranded cDNA synthesized using A431 poly(A)⁺ RNA as template, was ligated to *Cla*I linkers and inserted into the *Cla*I site of pBR322. Out of the ~10,000 clones screened, six hybridized to a 0.5-kbp *v-erb-B* *Bam*HI fragment (pE3, 4, 5, 6, 7, 8). One of these clones (pE7) had a 2.4-kbp DNA insert flanked at both ends by *Cla*I restriction sites. A second (pE3) contained a 1.3-kbp DNA *Cla*I insert. Homology between pE7 and the other cloned cDNAs was demonstrated by hybridizing ³²P-labelled pE7 insert DNA to other blotted plasmid DNAs.

The sequence of the 2.4-kbp pE7 insert (Fig. 1) contains one open reading frame which converts to ~790 amino acids; the carboxy-terminus exhibits great homology to the *v-erb-B* protein sequence (Fig. 1), and limited similarity to RSV p60^{src} (ref. 30). In addition, three of the six EGF receptor peptides sequenced by Downward *et al.*¹ match exactly with the deduced amino acid sequence of pE7 (Fig. 1, boxed sequences). The other three are not contained in the 2.4-kbp insert because during the cloning of this DNA a natural *Cla*I site within the original cDNA molecule (now representing the 3' end of the pE7 insert) was cleaved by *Cla*I before ligation into pBR322, thereby removing the extreme 3' portion of the cDNA.

It is not yet clear whether the *c-erb-B* gene product and the EGF receptor are identical or simply related proteins. In principle, this pE7 cDNA clone could be derived from mRNAs encoding either the human *c-erb-B* gene product or the EGF receptor. We believe that pE7 codes for the EGF receptor because of the structural similarity between the pE7 deduced amino acid sequence and the EGF receptor peptides, and because RNA levels quantified by hybridization with pE7 correlate extremely well with EGF receptor protein levels in a variety of cell types (Y.-h.X. *et al.*, in preparation).

An interesting feature of the pE7 sequence is a hydrophobic stretch of 27 amino acids (Fig. 1, darkly underlined) of which 20 are nonpolar and 7 are neutral; this is probably the hydrophobic transmembrane region of the EGF receptor. The putative site for a phosphoacceptor is shown in the sequence (Fig. 1, tyrosine •). In addition, there are ten potential *N*-glycosidic linkage sites (Asn-Met-Ser; Asn-Pro-Thr; Asn-Ala-Thr; Asn-Cys-Thr; Asn-Arg-Thr; Asn-Ile-Thr; Asn-Val-Ser; Asn-Ile-Thr; Asn-Asn-Thr; Asn-Cys-Thr; Fig. 1, lightly underlined)^{32,33} which could be involved in the complex glycosylation that occurs on the extracellular portion of the EGF receptor^{34,35}. Mayes and Waterfield³⁶ have reported that biosynthesis of the human EGF receptor in A431 involves co-translational addition of at least seven *N*-linked oligosaccharide chains. It is possible that most or all of the potential sites derived from this cDNA sequence are used in EGF receptor processing.

The fact that there exists one full-length open reading frame suggests that this cDNA was synthesized from a single mRNA molecule, and not formed from a recombinational event between two different cDNAs in the same vector. The ability of the insert to be cut out of the vector cleanly by *Cla*I also supports the likelihood of a normal recombinant plasmid construction. To prove that this long cDNA was made from a single mRNA, R-looping was performed using *Sal*I-linearized pE7 (no sites in the insert) and A431 mRNA. Analysis of numerous R-loops revealed that the extreme 5' end of the cDNA insert was consistently associated with RNA (long tail of pBR322); however, some variability in RNA hybridization was detected at the 3' end (short tail of pBR322). The data suggest that at least two categories of RNA-DNA hybrids exist (see Fig. 2 legend). One type of RNA forms a full-length hybrid, with colinear homology over the entire span of the insert (Fig. 2A). Many of these full-length RNA-DNA structures have RNA tails at both ends, suggesting that a significantly longer RNA is responsible for the R-loop formation. This RNA probably

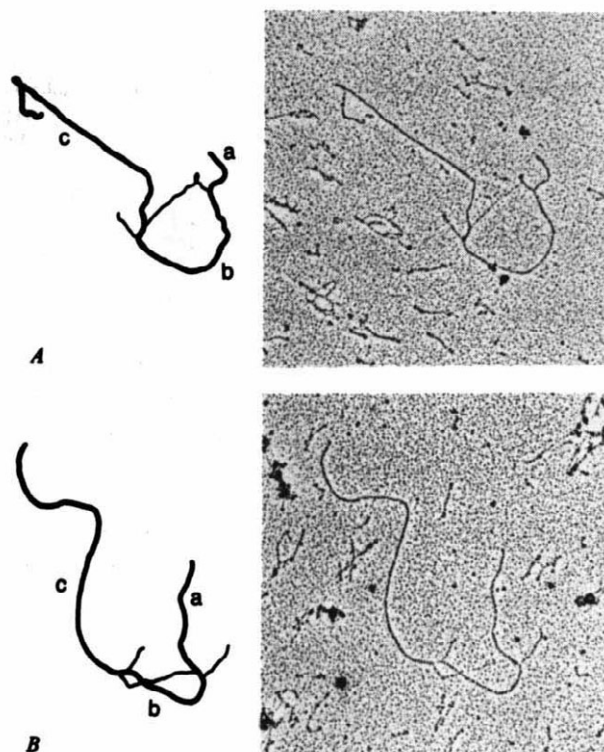


Fig. 2 R-loop analysis of pE7 using A431 poly(A)⁺ RNA. Purified plasmid pE7 was linearized by *Sal*I digestion (cuts once in pE7 within the pBR322 portion), and hybridized with poly(A)⁺ RNA. The hybrid molecules were spread and analysed⁵⁵. a, Short arm of pBR322; b, RNA-DNA hybrid; c, long arm of pBR322. The thin strand in the b region represents the noncomplementary single-strand insert DNA. A, B, Representative molecules which fall into two classes of R-loops. A, The average size and standard deviation (in bp) of the sections represented by a, b and c are 646 ± 223 , $1,673 \pm 250$, and $3,570 \pm 415$, respectively. Although only a 5' RNA tail is seen in this R-loop, other R-loops showed tails at either the 3' end, the 5' end, or both. B, The average sizes are $1,312 \pm 99$, $1,293 \pm 171$ and $3,583 \pm 294$. $\times 16,000$.

represents the normal EGF receptor mRNA, which would contain the coding region homologous with previously sequenced EGF receptor peptides, and which would have additional RNA regions extending beyond the cDNA insert at both ends of the molecule.

A second RNA forms an R-loop that is ~600 nucleotides shorter at the 3' end (Fig. 2B). This structure, which also appears to have tails at both ends, is seen more often than the longer R-loop described above. The frequent appearance of the shorter RNA-DNA hybrid molecule suggests that a second RNA exists in A431 cells which has strong homology to the 5' end of the pE7 insert, but not to the 3' end.

If the 5' end of the pE7 insert alone was capable of hybridizing to a different species of RNA, as suggested by the R-loop data, then Northern analysis using 3'- and 5'-specific pE7 cDNA fragments should confirm this. Messenger RNA was isolated from A431 and A498 cells (a human kidney carcinoma cell line which has about one-third the level of EGF receptors as A431; N. Richert and I.P., unpublished data), electrophoretically fractionated on 1% agarose, transferred to nitrocellulose, and hybridized to various cDNA probes. Figure 3 (lanes a) shows that the nick-translated 0.5-kbp *Bam*HI *v-erb-B* probe used to screen the cDNA library hybridizes to two prominent A431 RNAs of ~10 kb and 5.6 kb. Hybridization to A498 RNA gave a 4–5-fold less intense signal (Fig. 3b). Hybridization of the human pE7 probe to A431 RNA blots revealed a variety of

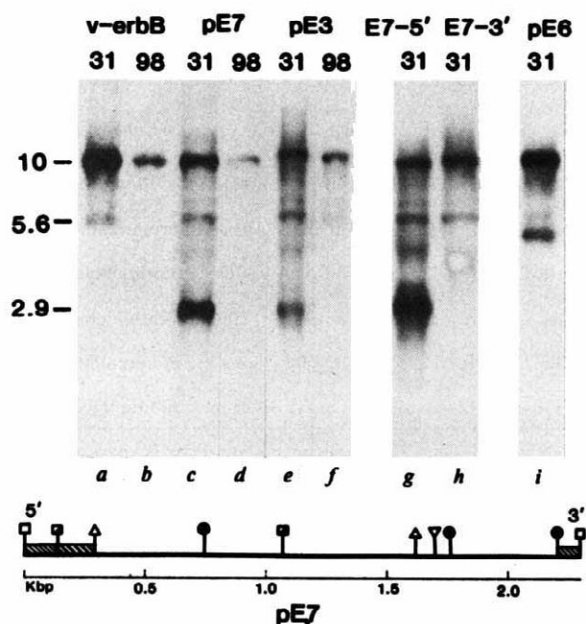


Fig. 3 Northern analysis of EGF receptor RNA. Poly(A)⁺ RNA was isolated as described in Fig. 1 legend, either from A431 cells (31, lanes a, c, e, g–i) or A498 cells (98, lanes b, d, f), fractionated on 1% agarose, and transferred to nitrocellulose³⁶. Nick-translated probes include the 0.5-kbp *Bam*HI *v-erb-B* fragment (lanes a, b); the 2.4-kbp cDNA insert from pE7 (lanes c, d); the 1.3-kbp insert of pE3 (lanes e, f); the 5'-specific *Cla*I–*Pst*I pE7 fragment (lane g); the 3'-specific *Bam*HI–*Cla*I pE7 fragment (lane h); and the 600-bp pE6 insert (lane i). Hybridization was in 50% formamide, 5×SSC at 42°C for 36 h. Washing included 1 h in 0.2×SSC, 0.1% SDS at 45°C. Numbers at the left indicate the size of the major RNA species in kilobases. Sizes were determined using rRNAs as markers, and the size of the 10 kb species is therefore an estimation. The restriction endonuclease map of the EGF receptor cDNA insert of pE7 is shown at the bottom. The region of pE7 corresponding to the *v-erb-B* 0.5-kbp probe is located approximately at the 1.75–2.25-kbp *Bam*HI–*Bam*HI fragment (see Fig. 1). The pE3 insert is located from ~1.0–2.3 kbp and the pE6 insert from 1.8 to 2.4 kbp on the map of pE7. The locations of fragments used for 5'- and 3'-specific hybridization are indicated by lined boxes. Restriction enzymes include *Cla*I (□), *Eco*RI (▽), *Pst*I (△), *Bam*HI (●) and *Pvu*II (■). A more detailed restriction map is available from the authors on request.

RNAs, including the 10-kb and 5.6-kb species (Fig. 3c). In addition, three minor RNAs (6.3, 4.6 and 3.3 kb) and a prominent 2.9-kb species were found in the A431 cells. These RNAs were all present in elevated levels in A431 cells relative to A498 cells (Fig. 3c, d). This is consistent with the possibility that many, if not all, of the RNAs are related to the EGF receptor. It seems likely that these RNAs are generated by either complex splicing, or are transcribed from a gene family with multiple members localized on several chromosomes in A431 cells.

When the 1.3-kbp insert of pE3 was used as probe, the same RNAs were found (Fig. 3e, f). However, hybridization with a pE6 probe which contains a small ~600-bp cDNA insert homologous to the 3' end of pE7 failed to visualize the 2.9- and 3.3-kb RNA species in A431 cells (Fig. 3i). The same result was obtained when a 90-bp fragment from the 3' portion of the pE7 was used as a probe to hybridize to A431 RNA blots (Fig. 3h). However, a 290-bp fragment from the 5' portion of pE7 hybridized to all the species detected by full-length pE7, including the 2.9- and 3.3-kb RNAs (Fig. 3g). The smaller 2.9-kb RNA is a relatively prominent species in A431 cells and is probably the species exhibiting great homology to ~1.5–1.6 kbp of the 5' portion of the pE7 insert (R-loop analysis, Fig. 2B); it therefore could code for a truncated form of the EGF receptor. Recently, it has been reported that A431 cells secrete a truncated EGF

receptor-like protein of the molecular weight 95,000–105,000 (refs 36, 37). This protein could be translated from a small A431 RNA (2.9 or 3.3 kb RNA). A 16-amino acid clostripain fragment of this truncated protein recently sequenced by Weber *et al.*³⁷ matches exactly with a stretch of amino acids deduced from clone pE7 at nucleotide positions 926–974 (Fig. 1). This region of the cDNA is homologous to the small RNA species as well as the large RNA (Figs 2, 3). These data confirm the close relationship between the truncated form and the full-length EGF receptor, and is consistent with the notion that one or both of the small RNAs encode the short receptor-like protein.

We recently reported that DNA sequences for the EGF receptor were amplified 30-fold and possibly rearranged in A431 cells³⁸. Shimizu *et al.*³⁹ have reported that in addition to two normal chromosomes 7 that contain the EGF receptor gene, A431 cells contain two marker chromosomes (M4 and M14) originating from translocations between chromosome 7 and unidentified segments of DNA. One of these (M4) confers high EGF-binding capacity to fused A431/mouse A9 cells. These data raise the possibility that the 10-kb and/or the 5.6-kb RNA are transcribed from a normal EGF receptor gene on chromosome 7, and the 2.9-kb RNA species is transcribed from a chromosome involved in a rearrangement event at or near the location of the EGF receptor gene in A431 cells (possibly M4 and/or M14). However, a new RNA species could be generated by any gene disruption causing alterations in the normal start site, stop site, or patterns of RNA splicing. Whether or not this RNA encodes a protein which is responsible for the elevated EGF-binding ability of A431 cells remains to be established.

An additional interesting finding concerns heterogeneity between the different cDNA clones, as determined by restriction mapping and partial sequence analysis (data not shown). Further work is needed to determine whether this represents differential RNA splicing of an unprocessed EGF receptor gene product.

One striking characteristic of the EGF receptor gene is its similarity to the *v-erb-B* gene¹. Because of the apparent functional relationship between growth factor receptors and oncogene products, it is interesting to compare our results for the EGF receptor with those involving other oncogene proteins. Transformed cells expressing high levels of either *myc* or *myb* protein were found to transcribe RNAs of unusual size, and to contain chromosomes involved in translocation events^{40–43}. One possible explanation is that the overproduction of the 2.9-kb RNA and its corresponding modified protein product may be associated with the appearance of the malignant phenotype of A431 cells. To address this question, and to elucidate the nature of a putative EGF receptor gene rearrangement in A431 cells, we are now characterizing cDNA clones encoding the 2.9-kb RNA.

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Note added in proof: Since submitting this paper, two reports^{57,58} reaching essentially the same conclusions, have been published.

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- Downward, J. *et al.* *Nature* **307**, 521–527 (1984).
- Fabricant, R. N., DeLarco, J. E. & Todaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 565–569 (1977).
- Savage, C. R. & Cohen, S. *J. biol. Chem.* **247**, 7609–7611 (1972).
- Holley, R. W. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2840–2841 (1972).
- Hollenberg, M. D. & Cuatrecasas, P. *J. biol. Chem.* **250**, 3845–3853 (1975).
- Barnes, D. & Colowick, S. P. *J. cell. Physiol.* **89**, 633–640 (1976).
- Cohen, S., Ushiro, H., Stoscheck, C. & Chinkens, M. *J. biol. Chem.* **257**, 1523–1531 (1982).
- Covelli, L., Mozzl, R., Rossi, R. & Frati, L. *Hormones* **3**, 183–191 (1972).
- Hollenberg, M. D. & Cuatrecasas, P. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2964–2968 (1973).
- Lembach, K. J. *J. cell. Physiol.* **89**, 277–288 (1976).

11. Das, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 2790-2794 (1977).
12. Sahyoun, N., Hock, R. A. & Hollenberg, M. D. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1675-1679 (1978).
13. Aharonov, A., Passovoy, D. S. & Herschman, H. R. *J. supramolec. Struct.* **9**, 41-45 (1978).
14. Hock, R. A., Nexø, E. & Hollenberg, M. D. *Nature* **277**, 403-405 (1978).
15. Cohen, S., Fava, R. A. & Sawyer, S. T. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6237-6241 (1982).
16. Cuatrecasas, P. & Hollenberg, M. D. *Adv. Protein Chem.* **30**, 251-451 (1976).
17. Aharonov, A., Pruss, R. M. & Herschman, H. R. *J. biol. Chem.* **253**, 3970-3977 (1978).
18. Wrann, M. M. & Fox, C. F. *J. biol. Chem.* **254**, 8083-8086 (1979).
19. Carpenter, G. & Cohen, S. A. *Rev. Biochem.* **48**, 193-216 (1979).
20. Beguinot, L., Lyall, R., Willingham, M. C. & Pastan, I. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2384-2388 (1984).
21. Engelbreth-Holm, J. & Rothe-Meyer, A. *Acta path. microbiol. scand.* **12**, 352-377 (1935).
22. Graf, T., Royer-Pokora, B., Schubert, G. E. & Beug, H. *Virology* **71**, 423-433 (1976).
23. Duesberg, P. H. *Cold Spring Harb. Symp. quant. Biol.* **44**, 13-30 (1980).
24. Bishop, J. M. *Cell* **23**, 5-6 (1981).
25. Hayward, W. S., Neel, B. G. & Astrin, S. M. *Nature* **290**, 475-479 (1981).
26. Klein, G. *Nature* **294**, 313-318 (1981).
27. Bishop, J. M. A. *Rev. Biochem.* **52**, 301-354 (1983).
28. Kondo, I. & Shimizu, N. *Cytogenet. Cell Genet.* **35**, 9-14 (1983).
29. Spurr, N. K. *et al. EMBO J.* **3**, 159-163 (1984).
30. Yamamoto, T. *et al. Cell* **35**, 71-78 (1983).
31. Privalsky, M. L., Ralston, R. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 704-707 (1984).
32. Beeley, J. G. *Biochem. biophys. Res. Commun.* **76**, 1051-1055 (1977).
33. Hanover, J. A. & Lennarz, W. J. *Archs Biochem. Biophys.* **211**, 1-19 (1981).
34. Hayman, M. J., Ramsay, G. H., Savin, K. & Kitchener, G. *Cell* **32**, 579-588 (1983).
35. Privalsky, M. L., Sealy, L., Bishop, J. M., McGrath, J. P. & Levinson, A. D. *Cell* **32**, 1257-1267 (1983).
36. Mayes, E. L. V. & Waterfield, M. D. *EMBO J.* **3**, 531-537 (1984).
37. Weber, W., Gill, G. N. & Spiess, J. *Science* **224**, 294-297 (1984).
38. Merlino, G. T. *et al. Science* **224**, 417-419 (1984).
39. Shimizu, N., Kondo, I., Gamou, S., Behzadian, M. A. & Shimizu, Y. *Somatic Cell molec. Genet.* **10**, 45-53 (1984).
40. Marcu, K. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 519-523 (1983).
41. Schwab, M., Alitalo, K., Varmus, H. E., Bishop, J. M. & George, D. *Nature* **303**, 497-501 (1983).
42. Mushinski, J. F., Bauer, S. R., Potter, M. & Reddy, E. P. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1073-1077 (1983).
43. Mushinski, J. F., Potter, M., Bauer, S. R. & Reddy, E. P. *Science* **220**, 795-798 (1983).
44. Smart, J. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 6013-6017 (1981).
45. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294-5299 (1979).
46. Fagan, J. B., Pastewka, J. V., Park, S. S., Guengerich, F. P. & Gelboin, H. V. *Biochemistry* **24**, 6574-6580 (1982).
47. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning*, 595 (Cold Spring Harbor Laboratories, New York, 1982).
48. Maniatis, T., Kee, S. G., Efstratiadis, A. & Kafatos, F. C. *Cell* **8**, 163-182 (1976).
49. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
50. Thuring, R. W. J., Sanders, J. P. M. & Borst, P. *Analyt. Biochem.* **66**, 213-217 (1975).
51. Messing, J. & Vieira, J. *Gene* **19**, 269-276 (1982).
52. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. *J. molec. Biol.* **143**, 161-178 (1980).
53. Anderson, S., Gait, M. J., Mayol, L. & Young, I. G. *Nucleic Acids Res.* **8**, 1731-1743 (1980).
54. Staden, R. *Nucleic Acids Res.* **4**, 4037-4051 (1977); **5**, 1013-1015 (1978); **6**, 2601-2610 (1979).
55. Word, C. J., Mushinski, J. F. & Tucker, P. W. *EMBO J.* **2**, 887-898 (1983).
56. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
57. Ullrich, A. *et al. Nature* **309**, 418-425 (1984).
58. Lin, C. R. *et al. Science* **224**, 843-848 (1984).

Generation of β -globin by sequence-specific proteolysis of a hybrid protein produced in *Escherichia coli*

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High-level expression of many eukaryotic genes has proved difficult to achieve even when a strong promoter¹⁻³ and the ribosome binding sequence^{4,5} from highly expressed *Escherichia coli* genes have been placed in front of the coding sequences. To overcome this problem, many eukaryotic proteins have been efficiently produced as hybrids after fusion of their genes with a coding sequence of *E. coli* genes⁶. However, such hybrid proteins are not suitable for functional studies or clinical use unless the authentic protein sequence can be released by specific cleavage. Here, we have inserted the sequence Ile-Glu-Gly-Arg between the 31 amino-terminal residues of λ cII protein and Val 1 of human β -globin, and produced this hybrid in high yield in *E. coli*. We then cleaved the hybrid specifically at the single arginine, using blood coagulation factor X_a and thus liberated the authentic β -globin chain. As factor X_a is specific for the tetrapeptide Ile-Glu-Gly-Arg⁷, which is rare in protein sequences, our expression/cleavage system is applicable to the efficient production of many eukaryotic proteins.

Table 1 Peptide bonds known to be cleaved by blood coagulation factor X_a

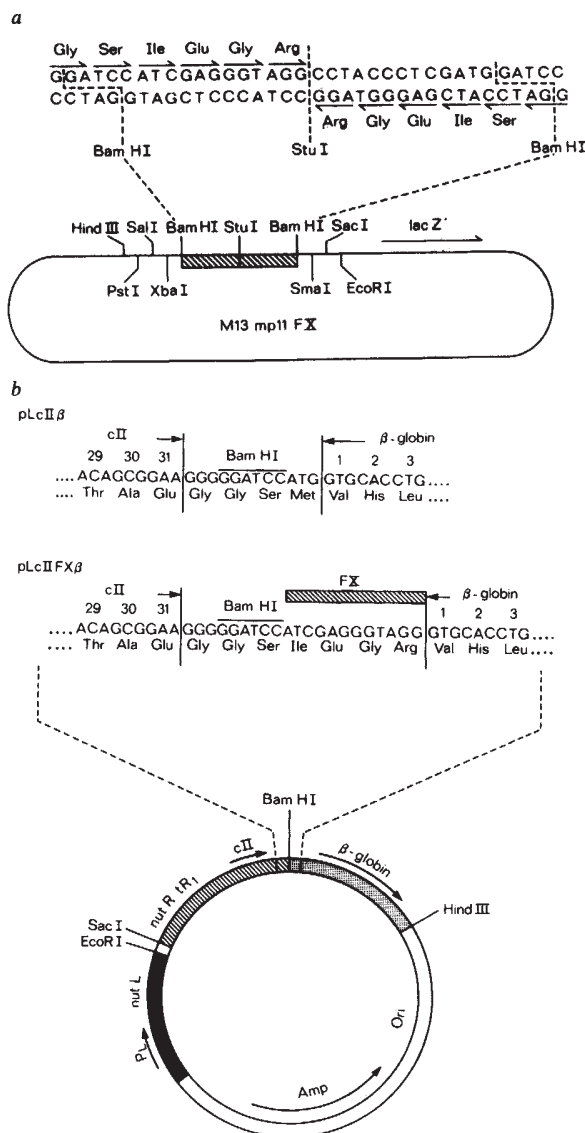
P ₄	P ₃	P ₂	P ₁	P' ₁	P' ₂	P' ₃	P' ₄	Substrate
Ile	-	Glu	-	Gly	-	Arg	=	Val - His - Leu - Thr
Ile	-	Glu	-	Gly	-	Arg	=	Thr - Ala - Thr - Ser
Ile	-	Glu	-	Gly	-	Arg	=	Thr - Ser - Glu - Asp
Ile	-	Asp	-	Gly	-	Arg	=	Ile - Val - Glu - Gly
Ile	-	Glu	-	Gly	-	Arg	=	Ile - Val - Glu - Gly
Ala	-	Glu	-	Gly	-	Arg	=	Asp - Asp - Leu - Tyr

Proteolytic cleavage by blood coagulation factor X_a occurs between P₁ and P'₁ sites.

* Factor X_a cleaves identically both native prothrombin and its chymotryptic peptides containing these peptide segments.

We first constructed a new expression vector, pLcII β , which directs synthesis of a hybrid protein consisting of the 31 amino-terminal residues of λ cII protein and the complete β -globin chain under the control of the λ P_L promoter (Fig. 1b). A secondary structure near the ribosome binding sequence should be retained even after the β -globin gene has been fused and should provide high translational efficiency. The control plasmid pLcII is identical to pLcII β , except that it contains a sequence with multiple restriction sites instead of the β -globin gene insert. When MZ-1 cells (a defective λ lysogenic strain) harbouring pLcII β are grown at 30 °C, the cI857 temperature-sensitive repressor is active and hence transcription from the P_L promoter is fully repressed^{8,9}, but at 42 °C the cII β -globin gene is efficiently transcribed. Figure 2 shows a Coomassie blue-stained polyacrylamide gel of total cellular proteins from cultures with and without heat induction. After heat induction, a new intense band appeared for MZ-1 cells harbouring pLcII β . The band co-migrated with soybean trypsin inhibitor consisting of 181 amino acid residues, the same number as in the cII β -globin hybrid. Western blot experiments¹⁰ showed that the new band, as well as natural human β -globin, reacted with ¹²⁵I-labelled goat anti-rabbit β -globin IgG (data not shown), indicating that the new band is the cII β -globin hybrid.

In a second stage, we inserted a short oligonucleotide between the λ cII and β -globin genes of pLcII β to form pLcIIFX β , so that the first codon (Val) of the β -globin gene was preceded by a sequence encoding the tetrapeptide: Ile-Glu-Gly-Arg (Fig. 1b). This is the sequence that immediately precedes the two factor X_a cleavage sites in prothrombin⁷. The hybrid protein, designated cIIFX β -globin, was purified from MZ-1 cells harbouring pLcIIFX β and digested with blood coagulation factor X_a which had been activated with Russell's viper venom¹¹. Digestion was complete within 2 h at a molar ratio of enzyme to substrate of ~1:100. No further cleavage occurred, except for a slow proteolysis by *E. coli* enzymes which occurred even in the absence of factor X_a. The new band that appeared after factor X_a digestion co-migrated with the slower band of haemoglobin corresponding to the β -chain (Fig. 3). In contrast, the cII β -globin protein which lacks the tetrapeptide insert remained uncleaved by factor X_a. Reverse-phase HPLC of tryptic peptides confirmed that the product of factor X_a digestion of cIIFX β -globin is identical to natural β -globin (data not shown). Cleavage of the cIIFX β -globin protein by factor X_a occurred exclusively at the peptide bond following Ile-Glu-Gly-Arg, even though the hybrid protein contained 6 other arginyl and 14 lysyl residues. In the same conditions trypsin cleaves β -globin at every peptide bond following arginine and lysine, thus the absence of such cleavage by coagulation factor X_a cannot be due to inaccessibility of the substrate. Moreover, the enzyme



does not require the three-dimensional structure of the substrate to be intact, as it cleaves the recognition sequences in both native prothrombin and in two chymotryptic prothrombin peptides⁷; this shows that it is strictly sequence-specific.

All the polypeptides cleaved by factor X_a contain arginine and glycine at the P₁ and P₂ sites, respectively, and the P₃ site is occupied by a negatively charged residue—glutamic acid or aspartic acid (Table 1). As the peptide bond following Leu-Gly-Arg in cIIFXβ-globin is not cleaved by factor X_a, a Glu-Gly-Arg or an Asp-Gly-Arg sequence seems to be a minimal requirement for cleavage. The requirement for the P₄ site is not yet clear, but the present limited data suggest that it must be occupied by a hydrophobic residue. Table 1, together with the data for other trypsin-like serine proteases, shows that the P₁' site can be occupied by a variety of amino acid residues, including a large aliphatic one, a positively charged one or a negatively charged one. On the other hand, trypsin-like proteases do not cleave peptide bonds preceding proline. Therefore, factor X_a will cleave any peptide bond preceded by Ile-Glu-Gly-Arg or Ile-Asp-Gly-Arg, unless proline occupies the P₁' site, and may be regarded as a 'restriction endopeptidase'. Several short eukaryotic polypeptides have been liberated from hybrid proteins by trypsin or cyanogen bromide, but such methods^{6,12,13} cleave proteins like β-globin at several arginines and lysines or at several methionines.

In our expression/cleavage system the bacterial leader sequence provides a high translational efficiency while the factor

Fig. 1 Construction of M13mp11 FX and pLcIIFXβ. **a**, M13mp11 FX contains a sequence encoding the recognition site for factor X_a. This M13 derivative can be used to join any coding sequence to the factor X_a recognition sequence. **b**, pLcIIFXβ and pLcIIβ direct efficient production of a hybrid protein consisting of the 31 amino-terminal residues of the λ cII protein and the complete human β-globin, with and without the factor X_a cleavage site, respectively.

Methods: All DNA manipulations were essentially as described elsewhere¹⁸. A temperature-sensitive lysogenic strain MZ-1 (*galK_{am}Δ8attΔBamN₇N₃₃c1857ΔH1, his⁻, ilv⁻, bio⁻, N⁺*, given by Dr K. McKenney) was used as a host strain for plasmids containing the λ P_L promoter and transformation was done as described by Remaut *et al.*⁸. T4 DNA ligase was prepared from strain NM989 (refs 19, 20). Restriction enzymes were from New England BioLabs. **a**, M13mp11 FX: Two oligonucleotides, dTACCTCGATGGATC and dCATCGAGGGTAGGCC, were synthesized by a phosphotriester method on a controlled pore glass support²¹ and purified by HPLC²². The two oligonucleotides were allowed to anneal after phosphorylation with T4 polynucleotide kinase (P-L Biochemicals) and γ -³²PATP (3,000 Ci mmol⁻¹; Amersham) and ligated to form concatamers. The DNA was then digested with *Bam*HI and cloned into the dephosphorylated *Bam*HI site of M13mp11 (ref. 23) to yield M13mp11 FX which forms blue plaques in the presence of isopropyl-β-D-thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Sigma). **b**, Construction of pLcIIβ and pLcIIFXβ: The *Eco*RI-*Hind*III fragment containing the multi-restriction sites was cut out from M13mp10 (ref. 23) and ligated to *Eco*RI-*Hind*III-cut pLc245 (ref. 8) to form pLmp10. The 319-base pair (bp) *A*luI fragment containing the *nut*R₁, *t*R₁ sites³ and a part of the cII gene was cut out from pKG1805 (ref. 24) and cloned into the *Sma*I site of M13mp10 in the same orientation with respect to the β-galactosidase α-peptide gene. The *Eco*RI-*Hind*III fragment containing the λ DNA sequence was then cut out and cloned into the *Eco*RI-*Hind*III site of pLmp10 to yield pLcII. A complete human β-globin cDNA sequence was reconstructed by joining restriction fragments prepared from an incomplete cDNA clone (pJW102)²⁵ and a genomic DNA clone²⁶, and this was cloned into the *Sma*I-*Hind*III site in M13mp9. The M13mp9 β cDNA thus obtained was opened at the *Nco*I site, which is located at the initiation codon, and treated with Klenow DNA polymerase (Boehringer Mannheim) in the presence of 100 μM 4dNTP to obtain flush ends. The β-globin cDNA sequence was then cut out with *Hind*III and inserted into the *Bam*HI (filled-in)-*Hind*III site of pLcII so that the β-globin gene was fused to the λ cII gene in-phase via a small linker DNA derived from M13mp10. To construct pLcIIFXβ, M13mp9 β cDNA was opened with *Nco*I and 40 μg of DNA were treated with 200 U of mung bean nuclease (P-L Biochemicals) in 30 mM Na-acetate pH 4.6, 50 mM NaCl, 1 mM ZnCl₂, 5% glycerol at 0 °C for 10 min to remove the 5' protruding end. The β-globin cDNA sequence was cut out with *Hind*III and cloned into the *Stu*I-*Hind*III-cut M13mp11 FX. The DNA sequence was determined by the dideoxy chain termination method²⁷ to ensure that the first valine codon of the β-globin gene was preceded by the DNA sequence coding for Ile-Glu-Gly-Arg. Then, the *Bam*HI fragment containing a part of the β-globin sequence was cut out and cloned into *Bam*HI-digested pLcIIβ to form pLcIIFXβ.

X_a cleavage eliminates the extra amino-terminal methionine encoded by the initiation codon which is present in most eukaryotic proteins expressed in *E. coli* by conventional methods⁶. The pro-enzyme, factor X, is readily prepared from bovine blood plasma with a yield of 5 mg l⁻¹. Factor X is isolated biochemically pure and free from contaminating proteases after adsorption from plasma on insoluble barium salts, followed by one chromatographic step¹⁴. For the construction of other hybrid protein expression vectors, any blunt-ended coding sequence can be cloned into the *Stu*I site of M13mp11 FX which contains a sequence encoding the factor X_a recognition site (Fig. 1a). The gene, together with the factor X_a recognition sequence, can then be cut out from the M13 clone with *Bam*HI and cloned into the *Bam*HI site of pLcII, or indeed any hybrid protein expression system, so that the cleavable hybrid protein can be produced. This method is now being applied, in our laboratory, to the production in *E. coli* of several eukaryotic proteins.

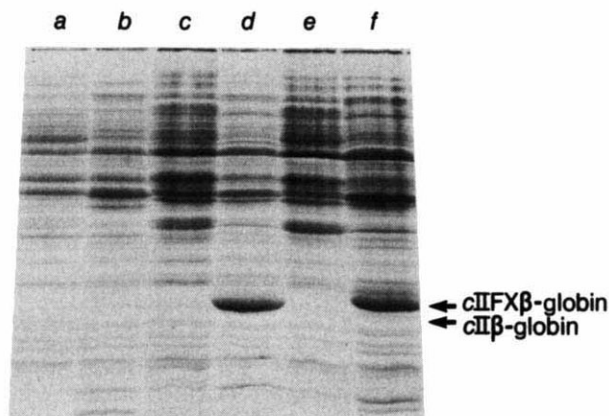


Fig. 2 Expression of cIIIFX β -globin hybrid protein in *E. coli*. Total cellular proteins from MZ-1 carrying pLcII, pLcII β and pLcIIIFX β , with and without 2 h of heat induction, were analysed on an 18% polyacrylamide-SDS gel and visualized with Coomassie blue. *a*, pLcII at 30°C; *b*, pLcII at 42°C; *c*, pLcII β at 30°C; *d*, pLcII β at 42°C; *e*, pLcIIIFX β at 30°C; *f*, pLcIIIFX β at 42°C. **Methods:** MZ-1 cells harbouring the expression plasmids pLcII, pLcII β and pLcIIIFX β were grown to $A_{600} = 0.7$ in 2 $\times$ TY medium (16 g Tryptone, 10 g yeast extract, 5 g l⁻¹ NaCl) at 30°C and for each of the cultures, one half was mixed with an equal volume of 2 $\times$ TY preheated to 65°C and grown at 42°C. The other half was grown further at 30°C as a control. The total cellular protein was extracted with an equal volume of phenol and spun down after precipitation with 5 vol. ethanol⁸. The pellet was dissolved in SDS sample buffer and analysed on an 18% polyacrylamide-SDS gel²⁸.

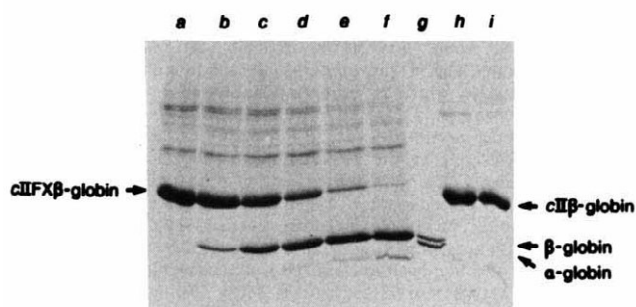


Fig. 3 Digestion of cIIIFX β -globin protein with bovine blood coagulation factor X_a. The cIIIFX β -globin hybrid protein was digested with factor X_a at an enzyme to substrate molar ratio of 1:100 at 25°C and analysed on an 18% polyacrylamide-SDS gel²⁸. *a*, Undigested cIIIFX β -globin; *b*, 5 min; *c*, 15 min; *d*, 30 min; *e*, 60 min; *f*, 120 min after addition of factor X_a; *g*, human adult haemoglobin comprising the α (faster band) and β (slower band) chains. The cII β -globin hybrid protein which lacks the cleavage site for factor X_a was treated with factor X_a in the same conditions: *h*, after 120 min of treatment; *i*, untreated cII β -globin.

Methods: MZ-1 cells harbouring pLcIIIFX β were grown in 500 ml of 2 $\times$ TY medium. At $A_{600} = 0.7$, the culture was mixed with 500 ml of 2 $\times$ TY preheated to 65°C, then grown at 42°C. After 2 h, cells were collected, and the high salt precipitate was prepared²⁹. The pellet was dissolved in 30 ml of 10 mM Na-phosphate pH 6.4, 1% SDS (BDH), 1% β -mercaptoethanol and incubated in a boiling water bath for 5 min. The sample was dialysed against 10 mM Na-phosphate pH 6.0, 1 mM dithiothreitol, 0.1% SDS and purified on a hydroxylapatite (Bio-Rad, DNA grade) column³⁰. The cIIIFX β -globin hybrid protein was concentrated by ultrafiltration (Amicon, PM-10 membrane) then precipitated with 6 vol. acetone/0.1 M HCl to remove SDS. The precipitate was air-dried, dissolved in a minimal amount of 8 M urea, 50 mM Tris-HCl pH 8.0 and dialysed against 100 mM NaCl, 50 mM Tris-HCl pH 8.0 and 1 mM CaCl₂. Bovine blood coagulation factor X (given by Dr M. P. Esnouf) was activated to factor X_a with Russell's viper venom (Sigma)¹¹.

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1. Remaut, E., Tsao, H. & Friers, W. *Gene* **22**, 103-113 (1983).
2. Queen, C. *J. molec. appl. Genet.* **2**, 1-10 (1983).
3. Shatzman, A., Ho, Y.-S. & Rosenberg, M. in *Experimental Manipulation of Gene Expression* (ed. Inouye, M.) 1-14 (Academic, New York, 1983).
4. Shine, J. & Dalgarno, L. *Nature* **254**, 34-38 (1975).
5. Steitz, J. A. in *Biological Regulation and Development, 1: Gene Expression* (ed. Goldberger, R. F.) 349-399 (Plenum, New York, 1979).
6. Harris, T. J. R. in *Genetic Engineering Vol. 4* (ed. Williamson, R.) 127-185 (Academic, London, 1983).
7. Magnusson, S., Petersen, T. E., Sottrup-Jensen, L. & Claeys, H. in *Proteases and Biological Control* (eds Reich, E., Rifkin, D. B. & Shaw, E.) 123-149 (Cold Spring Harbor Laboratory, New York, 1975).
8. Remaut, E., Stanssens, P. & Fiers, W. *Gene* **15**, 81-93 (1981).
9. Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. *Nature* **272**, 414-423 (1978).
10. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350-4354 (1979).
11. Fujikawa, K., Legaz, M. E. & Davie, E. W. *Biochemistry* **11**, 4892-4898 (1972).
12. Itakura, K. *et al. Science* **198**, 1056-1063 (1977).
13. Shine, J., Fettes, I., Lan, N. C. Y., Roberts, J. L. & Baxter, J. D. *Nature* **285**, 456-461 (1980).
14. Fujikawa, K., Legaz, M. E. & Davie, E. W. *Biochemistry* **11**, 4882-4891 (1972).
15. Walz, D. A., Hewett-Emmett, D. & Seegers, W. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1969-1972 (1977).
16. Butkowski, R. J., Elion, J., Downing, M. R. & Mann, K. G. *J. biol. Chem.* **252**, 4942-4957 (1977).
17. Magnusson, S., Sottrup-Jensen, L., Petersen, T. E., Dudek-Wojciechowska, G. & Claeys, H. in *Proteolysis and Physiological Regulation* (eds Ribbons, D. W. & Brew, K.) 203-238 (Academic, New York, 1976).
18. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
19. Murray, N. E., Bruce, S. A. & Murray, K. *J. molec. Biol.* **132**, 493-505 (1979).
20. Tait, R. C., Rodriguez, R. L. & West, R. W. Jr *J. biol. Chem.* **255**, 813-815 (1980).
21. Sproat, B. S. & Bannmarth, W. *Tetrahedron Lett.* **24**, 5771-5774 (1983).
22. Gait, M. J., Matthes, H. W. D., Singh, M., Sproat, B. S. & Titmas, R. C. *Nucleic Acids Res.* **10**, 6243-6254 (1982).
23. Vieira, J. & Messing, J. *Gene* **19**, 259-268 (1982).
24. McKenney, K. thesis, Johns Hopkins Univ. (1982).
25. Wilson, J. T. *et al. Nucleic Acids Res.* **5**, 563-581 (1978).
26. Lawn, R. M., Efstratiadis, A., O'Connell, C. & Maniatis, T. *Cell* **21**, 647-651 (1980).
27. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
28. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
29. Gilmer, T. M., Parsons, J. T. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2152-2156 (1982).
30. Moss, B. & Rosenblum, E. N. *J. biol. Chem.* **247**, 5194-5198 (1972).

Erratum

Expression and amplification of the N-myc gene in primary retinoblastoma

W.-H. Lee, A. L. Murphree & W. F. Benedict
Nature **309**, 458-460 (1984).

THE first paragraph on page 460 was printed incorrectly. It should read: 'amplification in neuroblastoma and N-myc expression in the retinoblastoma strongly suggests that the N-myc gene may belong to the proto-oncogene family. The fact that the N-myc gene is amplified and expressed in both retinoblastoma and neuroblastoma is of obvious interest. This is especially so because both tumours originate from neural crest ectodermal cells, although retinoblastoma may develop from a more primitive cell than neuroblastoma²². However, we have no evidence to exclude the possibility that genes other than N-myc are involved in these two types of tumour formation.'

Science for sale

Dorothy Nelkin

The New Politics of Science. By David Dickson.
Pantheon, New York: 1984. Pp.374. \$22.95.

THAT knowledge is power has been well understood and much discussed in many analyses of the politics of science. In *The New Politics of Science*, David Dickson describes the new dimensions of this truism in the 1980s, arguing that science today is a commodity, a policy instrument, a source of legitimation, a basis of imperialism in foreign policy, and a form of property that is being manipulated according to the needs of the military, the marketplace and the private sector. It is a provocative book. Dickson, the European correspondent for the journal *Science*, first characterizes a set of systematic and interrelated changes in American policies concerning science and technology. He then turns to their implications for the nature of scientific research and structure of universities and, more important, to their influence on the relationship between the citizen and the state.

Dickson argues that science is increasingly used to reinforce the concentration of political power in the hands of those who dominate the economy. Tracing the development of patterns of control over science during different historical periods, he concludes that recent changes, reflecting an "almost religious belief in the power of science-based technology", are dramatic and potentially pernicious. Indeed, he has written this book with the openly political purpose of revealing the problematic political and social consequences of recent changes in science and technology policy in the United States. Because of his concentration upon the influence of capitalism, some may interpret the book as merely a diatribe from the left. It is not. Rather, Dickson is pointing to a set of pressures which, taken together, indicate sweeping structural changes in the politics of science.

Dickson's foremost worry is that decisions about both the allocation of scientific resources and the application of scientific results are increasingly concentrated in the hands of corporate banking and military leaders who have gained the willing cooperation of scientific, governmental and academic institutions. He describes how the demands of the private sector influence government research policy and leave social objectives (such as protection of the environment) as issues secondary to the profitability of private corporations. He follows the changing character of budgetary decisions as science is increasingly viewed as "knowledge capital" expected to generate an appro-

priate return. And he suggests that unfettered technological development is endorsed in order "to get the economy back on its feet", with little attention being paid to its social consequences.

Dickson documents three striking manifestations of the changing politics of science. First are the new relationships between industry and academia that have developed with the renewed commercial interest in basic research. Knowledge, argues Dickson, is a "commodity", a form of "real estate". The result is that the public resources that universities represent are increasingly placed at the service of private capital.

Second, Dickson turns to the renewed links between the American scientific community and the military establishment, as reflected both in changing patterns of military funding of academic research and in growing security controls. Tracing the increased influence of the military in academia since the 1970s, he calls attention to the ironies of scientists accepting — and indeed soliciting — military support while worrying about controls on open communication as the government seeks to limit the transfer of technological information in the interests of national security.

Third, through numerous examples, he points to the growing foreign policy implications of science and technology, given their importance in international economics. Influenced by multi-national corporations, Third World politics are especially vulnerable to policies that are determined by profit rather than need. So too are international agreements such as the Law of the Sea.

Dickson finds it notable that today's politics of science developed after a decade of challenge and introspection during which many people, through the environmental and consumer movements, questioned the definition of "progress" and insisted that science and technology must serve the needs of society rather than determine those needs. That period of protest revealed two conflicting approaches to the politics of science based on "democratic" and "technocratic" strategies. The technocratic approach called for "rational" solutions to the problems of technology through a consensus of experts; the democratic strategy looked towards redistribution of political power through greater public involvement in technological decisions.

For a brief period, demands for

increased democratic control over technology appeared to be influencing the politics of science and technology as public agencies groped for assessment procedures that would involve citizens in general. The new politics of science represents a powerful counter-attack against such reforms. Today, Dickson argues, technocratic strategies prevail as technical and economic decision-making methodologies are used to cloak political judgements in the garb of scientific neutrality; and scientific arguments are used to dismiss challenges from concerned citizens. In effect, science has become a policy instrument reinforcing the ascendancy of technocratic over democratic values and reducing political choice.

What are the consequences of the new politics? Dickson sees a declining interest in the environmental, social and ethical implications of research. And as criteria of efficiency replace the emphasis on equity, he sees a re-direction of research away from social priorities. He also discerns changes within the community of science as commercial and military interests lead to secrecy and to tensions over traditional patterns of scientific communication. He worries about relationships within universities as certain departments compete to keep their faculty together in the face of well-paid entrepreneurial temptations. But, above all, Dickson worries about the political consequences of the new politics of science — the reduced possibilities for public involvement in political decision-making and the increased deference to technical expertise. Indeed, he sees the future of democracy itself as being at stake.

Dickson's argument is supported by many case-histories and placed in perspective with historical comparisons. While he is convincing, he also raises a number of unanswered questions. How did such dramatic political changes take place so quickly with so little public response? Why are academic scientists, who only a decade ago were widely disenchanted by university-military relationships, so quick to renew those relationships today? Why are campuses and grass-roots movements, until recently mobilized around related issues (military research in universities and the environmental and social effects of technical change), now so apathetic? How have the actors involved in the new politics of science cultivated the public apathy that has allowed uncritical deference to technocratic expertise? Dickson says too little about the media as a vehicle for gaining public consent for the new politics of science, and does little to explain the pressures within science that foster changing attitudes.

Dickson is by no means anti-science or opposed to technological change. Rather, he wants to re-integrate public needs and aspirations into science policy, to shift the priorities of science and technology away from destructive ends (such as defence) and

towards socially constructive goals (such as better health or nutrition). He concludes with some general proposals. He would like to democratize procedures within the scientific community so that priorities and practices would be open for broader public discussion; he would like to change the institutions responsible for allocating research funds to allow greater public input; he wants to develop ways of democratizing technological innovation to ensure that considerations of equity influence production; and he wants to maintain public access to the fruits of publicly funded research.

Dickson ends his book on a bizarre note, by taking encouragement from the effectiveness of the Creationists in challenging scientific ideas. In my view, he misinterprets the significance of

Creationism. Far from creating a "political program that includes a vision of an alternative science", this group has manipulated the populist tendencies of American Fundamentalists to enhance the power of a small religious group. Yet this conclusion to the book should not divert the reader from its very positive, indeed Utopian message — that science, one of the greatest cultural and intellectual achievements of the modern age, must be shaped in accordance with democratic values in order to enhance the public good. □

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Metallic electrons

Michael Springford

Magnetic Oscillations in Metals.

By D. Shoenberg.

Cambridge University Press: 1984.
Pp. 570. £55, \$97.50.

OCCASIONALLY a book appears of which it may be said that the author, the content and the timing could not be bettered. *Magnetic Oscillations in Metals* by Professor David Shoenberg is just such a book.

It was in 1932, at the suggestion of Kapitza, that the author, then a new graduate student at the Royal Society Mond Laboratory in Cambridge, was introduced to the mysterious magnetic properties of bismuth. Only a few years previously, in Leiden, de Haas and van Alphen had observed magnetic oscillations in this metal at low temperatures. The oscillations of the magnetization as the magnetic field was varied were thought at first to be peculiar to bismuth, but in time they were understood to be a fundamental property of all metals.

In a historical introduction, Shoenberg charts the course of the development of the de Haas-van Alphen effect, as it came to be called, from a curiosity to arguably the single most important experiment in solid state physics. We read of the insights of Landau on the origin of the effect, of the unexpected success of the nearly free electron model as crucial evidence in the development of pseudopotential theory, and of the subtle and fruitful interplay over the years between experiment and theory. The influence exerted on the course of solid state physics by studies of magnetic oscillations originated in large measure from their versatility and accuracy. Band theoretic methods, for example, could be fine-tuned and became more useful and reliable instruments in other fields. For more than half-a-century, in events from

which the author has seldom been far removed, magnetic oscillations have been employed as a spectrometer for the investigation of electrons in metals. It is with these many aspects of the field that the book is principally concerned.

An objective early in the book is to expound the theory of magnetic oscillations, broadly along the lines first set out by Lifshitz and Kosevich in 1955, use being made of the now-familiar geometrical interpretation of electronic properties for the physical insight it provides. The theory is developed for both two- and three-dimensional systems, with the more cumbersome mathematical results being helpfully placed in appendices. Given the historical development of the subject it is proper that the determination of Fermi surfaces (Fermiology), should have a prominent place. Here Shoenberg concentrates more on general principles, following this with a survey of what has been accomplished in a few selected metals. For a more encyclopaedic approach the reader is referred elsewhere.

In later chapters, attention is focused on more contemporary topics where, in characteristic style, the author does not fail to highlight areas where uncertainties remain, such as the effect of dislocations upon magnetic oscillations, nor those, such as the influence of many-body interactions, where even the general principles involved are still very much in question.

Although the study of magnetic oscillations has reached maturity as an established branch of solid state physics, this is remarkably the first book to be devoted to a complete exposition of the subject. Work on magnetic oscillations is still actively pursued and the author believes that this will continue for a long time yet. So too will his book remain the definitive text for research workers in this field. □

Michael Springford is Professor of Experimental Physics at the University of Sussex.

Cultural influence

Paula Brown Glick

Ruth Benedict: Patterns of a Life.

By Judith Modell.

Chatto & Windus/University of Pennsylvania Press: 1984. Pp. 355. Hbk £15, \$30; pbk \$11.95.

RUTH BENEDICT (1887–1948) has occupied an important place in the history of American anthropology for the past 50 years, ever since the publication of *Patterns of Culture* in 1934. Judith Modell, the author of this biography, is herself an anthropologist and quite clearly feels a strong personal identification with her subject. Yet she does not attempt to evaluate Benedict's scholarship or contribution to anthropology.

The biographer is able to cite Ruth Benedict's published and unpublished writings — poems, notebooks, letters and an autobiographical fragment written in 1935 — in her examination of Benedict's childhood and youth. But now, over 30 years after Benedict's death, recollections culled from friends, relatives and students do not fully satisfy inquiry — many questions remain. From the characterization of Stanley Benedict, her husband, and of some close friends, the figures remain shadowy; here the reader must accept the limitations of the biographer's materials. Modell has attempted to discern the pattern of Benedict's life, and has certainly laid out the whole sweep of her person and her interests, as child and adult, in literature and anthropology. The influence of philosophical and religious thought upon Benedict is not fully examined, however; her feminism is muted. And a chapter on her anthropological work was evidently unduly influenced by Margaret Mead's books about Benedict, and seems uncritically repetitive.

Ruth Benedict was a student of Franz Boas of Columbia University, who has been called the "builder and architect of modern anthropology" and who built the Anthropology Department at Columbia University into a centre for anthropology, ethnography and folklore study. Ruth Benedict entered this realm in 1921, in her thirties, there finding a personal role, a purpose for her life and a new subject for writing. She visited American Indian settlements to record texts and cultural data from informants — the usual method of American anthropologists of the time — and was Boas's closest assistant for 20 years, teaching and supervising students

New in paperback

● *A Feeling for the Organism: The Life and Work of Barbara McClintock* by Evelyn Fox Keller. (W.H. Freeman, \$8.95, £7.95.) For review see *Nature* 304, 377; 1983.

● *Darwin and His Critics* by David L. Hull. (University of Chicago Press, \$15, £12.75.) For review see *Nature* 248, 285; 1974.

although she had no permanent academic position at Columbia.

Boas's women students, especially Margaret Mead and Ruth Benedict, became widely known to American students and scholars in fields far beyond the small group of cultural anthropologists of the time. While Mead married and made many field trips with her husbands, Reo Fortune and Gregory Bateson, and later with students, Benedict remained at Columbia. There she edited the *Journal of American Folklore*, taught and published. Only after she separated from her husband and lived independently in New York did she have a regular post at the university.

Benedict's best-known work, *Patterns of Culture*, was the culmination of her interests in coherence of culture as a design for living. The work has long been recognized as an anthropological landmark. It developed from Boas's concern with ethnographic detail to the integration of a whole "culture". This notion that a culture, the total way of life of a people, could be characterized in a single word, Apollonian or Dionysian, was Benedict's unique insight; Modell feels it is her real achievement. Such a breathtaking whole view could not be achieved as a characterization of very many of the cultures studied by anthropologists, and this difficulty with the concept has been noted by a number of critics. However, the identification of themes and the relations between the individual and cultural values became one of the main lines of anthropological inquiry and had wide influence. At the beginning of the Second World War, Benedict and Mead, with a group of anthropologists and psychologists, carried out studies of the personality and culture of contemporary peoples, especially in European and Asian nations. The best-known of these studies, Benedict's *The Chrysanthemum and the Sword*, became a handbook used by American occupation forces in Japan after the Second World War.

Benedict died in 1948 at the age of 61, Mead and others completing the work on national character. Since the early 1950s, however, the attempt to identify a single cultural theme or pattern has been criticized as oversimplification. This, then, the outstanding contribution of Benedict to anthropology, was not followed up successfully by the next generation of scholars. Benedict's approach to anthropology as one of the humanities declined as cultural and psychological anthropology became more scientific in its methods and results.

The discipline of anthropology may indeed have progressed beyond the goal of identifying a unitary cultural theme. Nonetheless the person and concepts of Ruth Benedict, as presented here by Judith Modell, are of lasting interest and value to students of life and of culture. □

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States of mind and of science

Peter Wason

From Folk Psychology to Cognitive Science: The Case Against Belief.

By Stephen P. Stich.

MIT Press: 1984. Pp.266. \$22.50, £21.40.

THE ordinary reader might expect to learn from this book something about the so-called cognitive revolution which has transformed the science of psychology over the past few decades. After all, the title suggests something of this kind. Like folk medicine, folk psychology carries an unscientific, even a disreputable, connotation; it conjures up an image of untutored individuals who lack access to the sources of enlightenment. On the other hand, cognitive science has an honorific connotation, without the parochial aura of psychology and suggesting a lot of brainy people from some of the new disciplines (artificial intelligence, for example) all toiling together happily on fundamental epistemological problems and talking a language nobody else can understand.

The ordinary reader would not be entirely right. Dr Stich is not concerned with psychological research, but with a philosophical argument about the status of the mind and its cognitive capacities. His main concern is to propose that the concept of belief (and allied mental concepts) has no proper function in a scientific explanation of mental life. We have all got things wrong if we want to be taken seriously as scientists. Neither folk psychology, nor cognitive science, is accurately defined, but the former is assumed to comprise "a loose network of largely tacit principles, platitudes, and paradigms" based on mentalistic terms. The author's argument is that

such folksy notions, re-introduced into cognitive psychology after the bleak days of behaviourism, are detrimental to scientific progress and have no place in scientific accounts. This echoes in a strange way Otto Neurath's *International Encyclopedia of Unified Science* (1955), with its aim of purging scientific terms of surplus meaning. It is strange because positivism is hardly considered respectable in advanced philosophical circles and certainly not among the adherents of cognitive science. It is not simply in politics that left and right end up advocating the same thing.

Although the author draws back from giving whole-hearted assent to the claim of a couple of other philosophers that folk psychology is a "degenerating research program", he would like to rule out from cognitive science any "content sentence" which expresses an intentional or propositional attitude. Thus none of the following gain admittance:

S believes that Ouagadougou is the capital of Upper Volta
S hopes that Ouagadougou is the capital of Upper Volta
S fears that Ouagadougou is the capital of Upper Volta
S desires that Ouagadougou is the capital of Upper Volta,

for the folk psychological use of terms such as "believes", "hopes", "fears" and "desires" do not "play a role in the best and most sophisticated theories put forward by contemporary cognitive scientists". The precise nature of these admirable theories remains unspecified, but one might raise the question of how a clinician can make a diagnosis of paranoia without reflecting upon the beliefs of the patient. Further, one might also point out that there are philosophers who have made interesting analyses of emotions in ordinary folk terms.

What are the proposals which the author commends in the place of such crude

The Tables Turned: An Evening Scene

*Up! up! my friend, and clear your looks,
Why all this toil and trouble?
Up! up! my friend, and quit your books,
Or surely you'll grow double.*

*The sun above the mountain's head,
A freshening lustre mellow,
Through all the long green fields has spread,
His first sweet evening yellow.*

*Books! 'tis a dull and endless strife,
Come, hear the woodland linnet,
How sweet his music; on my life
There's more of wisdom in it.*

*And hark! how blithe the throstle sings!
And he is no mean preacher;
Come forth into the light of things,
Let Nature be your teacher.*

*She has a world of ready wealth,
Our minds and hearts to bless —
Spontaneous wisdom breathed by health,
Truth breathed by cheerfulness.*

*One impulse from a vernal wood
May teach you more of man;
Of moral evil and of good,
Than all the sages can.*

*Sweet is the lore which nature brings;
Our meddling intellect
Misshapes the beauteous forms of things;
— We murder to dissect.*

*Enough of science and of art;
Close up these barren leaves;
Come forth, and bring with you a heart
That watches and receives.*

This poem, by William Wordsworth, is taken from *Poems of Science*, a new anthology published by Penguin. The book is edited by John Heath-Stubbs and Phillips Salman, and among the poets represented are Shakespeare, Milton, Shelley, Walt Whitman, Louis MacNeice and John Updike. Price is £4.95 in paperback.

notions? His favoured option, carefully argued against other candidates, is "The Syntactic Theory of Mind" (STM). It is introduced in these words:

The basic idea of the STM is that the cognitive states whose interaction is (in part) responsible for behaviour can be systematically mapped to abstract syntactic objects in such a way that causal interactions among cognitive states, as well as causal links with stimuli and behavioural events, can be described in terms of the syntactic properties and relations of the abstract objects to which the cognitive states are mapped. More briefly, the idea is that causal relations among cognitive states mirror formal relations among syntactic objects. If this is right, then it will be natural to view cognitive state tokens as tokens of abstract syntactic objects.

I do not find this altogether intelligible, and not just because I fail to understand how objects can have a syntax. I do not really grasp how a theory such as this (however elaborated) could illuminate mental processes, and particularly the ways in which they go wrong in real life. However, it will be retorted (rightly) that the theory is not *really* a theory at all, but a kind of prolegomenon for a cognitive theorizing. One might suppose, then, that it is meant to be a methodological prerequisite for research, on the analogy with the methodologies for behaviourism which were proposed in the 1940s and 1950s. But there seems to me an interesting difference between them. The philosophers of science who analysed the concepts of behaviourism, as well as the concepts of the physical sciences, based their research on the use of the concepts within the theories. Philosophers of mind tend to ignore previous theory and appeal to their own insights, conjectures and intuitions, paraded forth in a series of pregnant sentences. There is nothing wrong with this, and it is often quite effective in advancing an argument. But the logical precision achieved in this way is also likely to be much more puzzling to the non-specialist than was the more discursive work of some of the great philosophers of the past.

There is a real danger that philosophers will end up talking only to each other and abandon their traditional role of protecting us from conceptual confusion. My doubt about the present book is whether its thesis will be of interest to experimentalists. It is a doubt rather than a verdict. Although Stich's arguments are nearly always compellingly precise, I am unsure whether they are consequential, or merely dazzling moves in an intellectual game. In the nature of things, they will not be readily settled because assertions about states of mind are not to be verified like assertions about nerve impulses. So while this book may be of interest to philosophers and to cognitive scientists, it remains uncertain whether it will help the rest of us in trying to find out more about mental processes. □

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Talk of research

Augustine Brannigan

Opening Pandora's Box: A Sociological Analysis of Scientists' Discourse.

By G. Nigel Gilbert and Michael Mulkay.
Cambridge University Press: 1984. Pp. 202.
Hbk £22.50, \$37.50; pbk £7.95, \$12.95.

OVER the past decade, a close descriptive approach to scientific behaviour has revived interest in the cognitive and social dimensions of contemporary scientific knowledge. Following in this direction, the Pandora investigation focused on a recent controversy in biochemistry. The study is based on intensive interviews with 34 bioenergists in the United States and Britain, as well as on a sample of their correspondence and a reading of the literature in the field between 1960 and 1980. This period witnessed the rise of competing theories to explain how the crucial ATP molecule was able to move and temporarily store energy in living cells. In 1978 Peter Mitchell was awarded a Nobel Prize for chemistry for his work on the role of oxidative phosphorylation in ATP formation.

However, research by the authors indicated that members of the "ox-phos" community, as it is called, were far from in agreement about what exactly had been discovered in chemiosmotic research, about the empirical validity of the various bits of evidence, and about the respective parts played by the leading contributors. In their travel between the various biochemical laboratories, Gilbert and Mulkay report they were struck by the fact that although they realized that they were being given radically different accounts by participants of what "really" had happened, each account seemed, on its own, plausible and convincing.

Rather than trying to find out who was "really" correct and who was in error, the sociologists, not being in the business of second-guessing biochemical questions, turned their attention to what they describe as "discourse analysis". This is what opens Pandora's Box. Discourse analysis requires that the sociological observer remains neutral as to who is "actually" correct, the object being to determine *how* the respective participants produce and maintain their own sometimes contradictory versions of reality in the face of what would seem to be a set of invariant natural facts.

The Pandora investigation was not undertaken as a critique of science, for Gilbert and Mulkay go well beyond observations of how scientists selectively expose themselves to natural facts and selectively perceive and retain them. The heart of their inquiry is the observation that scientists employ two repertoires or codes of discourse for talking about how nature is discovered. The familiar empiricist frame of mind explains progress as following nat-

urally from adherence to careful observation, and the testing of rival hypotheses in controlled experiments. In addition to this, scientists also employ what is called a "contingent" repertoire in which progress is traced to unique, subjective or situationally specific factors. Gilbert and Mulkay illustrate the use of these two conflicting repertoires in a fascinating contrast between the informal factors that influenced the investigation and interpretation of "ox-phos" experimental data reported to them in interviews, and the formal rationality provided in the earlier published articles of the same researchers. Rather than siding with the informal "inside" story as the true one, they instead stress that the accounts given of actions vary with the needs of the circumstance in which they are produced. Different accounts draw from the original experience in different ways depending on the formality or informality of the situation.

Having made a convincing case for the existence of two separate repertoires for reporting the progress of chemiosmotic theory, the authors then go on to suggest that these repertoires are employed to explain the differential acceptance of scientific beliefs across the community. While one would expect that consensus ought to follow closely the reading of the facts, this is not the case. The evidence shows that scientists typically ground their own beliefs in empiricist discourse, as following from the experimental results, while explaining the position of opponents in the contingent repertoire: opponents wrongly interpret the facts because of subjective bias, experimental incompetence, theoretical ignorance — and even national origins! This switching between the two repertoires virtually guarantees that opponents will talk past one another. According to the authors, the device which saves the community from solipsism is the belief that in the end "the truth will out" — though how, when and in whose favour, no one can determine beforehand.

Other highlights of this work include an outstanding analysis of the tension between the virtual versus the fictional elements in scientific drawings of the chemiosmotic mechanisms. One informant dubs them "working conceptual hallucinations". There are also discussions of consensus and the significance of scientific humour. Each of these topics is explored usefully in the context of the two-repertoire dichotomy. While based on a single scientific speciality, the lessons of this investigation probably have wide currency in all areas of science, especially where controversy mobilizes the two incommensurable structures of discourse identified here. For that reason the book will be read with profit by practising scientists as well as by sociologists of scientific knowledge. □

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Bags for waste, filters for hybridization

This month's new products include a filter holder for colony hybridization and autoclavable bags for the disposal of hazardous waste.

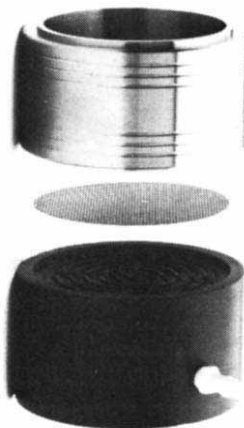
● The colony hybridization technique used in identifying bacteria in a large population which contains specific cloned nucleic acid fragments can be made simpler with the Schleicher & Schuell MV 08210 filter holder. Lysing, chloroform treatment, washing before and after hybridization, and hybridization itself can be carried out on the membrane-blotted replica colonies in the filter holder. Colonies are prevented from merging into each other when the holder is used. The apparatus is self-sealing and fitted with a 250 ml capacity stainless steel funnel, suitable for filter diameters of 82/87 mm. *Circle No. 100 on Reader Service Card.*

● The MS III is a sophisticated stimulator produced by World Precision Instruments Inc. It is microprocessor controlled and all stimulus parameters are entered using a numeric key pad. All keystroke entries are menu-prompted on a sixteen-character alpha/numeric display. Stimulus protocols may be assembled using any of eight pre-programmed or eight user-defined waveforms. MS III will store up to eight protocols in battery backed-up memory and may be interfaced with a computer via its RS232 port. *Circle No. 101 on Reader Service Card.*

● The Sera-Lab range of monoclonal antibodies now includes the mouse mono-PAP, monoclonal mouse peroxidase anti-peroxidase antibody (catalogue no. RP-2000). Like the rat mono-PAP already available, this gives a sensitive method with very low nonspecific background staining for demonstrating immunoreactive sites. *Circle No. 102 on Reader Service Card.*

● Vitatron Scientific B.V. of Dieren, the Netherlands have introduced a new series of photometers, called the IFP and the ISP. The IFP is fitted with a flexible cuvette system that can hold either four cuvettes or a Vitatron draincell, and which can be thermostatted through a waterbath. The ISP is equipped with a flow-cell for low volumes, suitable for routine analyses. Both photometers incorporate a complete range of procedures and calculations for both kinetic and endpoint measurements. Kinetic measurements can be carried out as either an integral of the curve with a check on linearity, or as two-point measurement. Results can be calculated using reagent blank or sample blank corrections. *Circle No. 103 on Reader Service Card.*

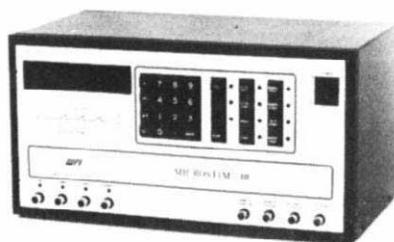
These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



A new filter holder (left) makes colony hybridization simpler, and the Omega CN2010 does the same for temperature control.

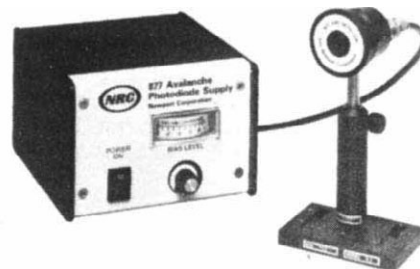
● The Newport Model 875 high-speed PIN photodetector has broad spectral sensitivity from 400 to 1,060 nm and sub-nanosecond risetime. The detection area is large and is easy to align in a beam. An optional optical fibre input is available. The Model 877 ultra-high speed APD photodetector has rise and fall times of less than 200 picoseconds. It is useful for characterizing laser diodes as it is designed to detect and resolve ultra-short pulses of visible light. Both feature rechargeable battery power supplies. *Circle No. 104 on Reader Service Card.*

● Marubishi bench-top cell cultivation fermentors for animal and mammalian cells are now available in the United States from Bioengineering Associates Inc. These fermentors are suitable for a wide range of cell types and methods of cell cultivation, including cell suspension, microcarriers, hybridoma and plant cells. Agitation is produced gently by a marine-type propeller driven by magnetic coupling to eliminate any penetration of the fermentation chamber. Automatic temperature control within 0.5°C is achieved by a proportional controller balancing the output of an electric heating unit with the flow of a cool water circulating system. *Circle No. 105 on Reader Service Card.*



● New from Pharmacia P-L Biochemicals, "Gene cartridges" are a group of promoters, antibiotic resistance genes and multi-site linkers used to custom-tailor cloning vectors to the customer's specifications. The range includes cloning vectors, oligonucleotides, molecular biology enzymes and restriction nucleases, all listed in the Pharmacia PL "Product Reference Guide". *Circle No. 106 on Reader Service Card.*

● The new Omega CN-2010 programmable temperature controller features a "ramp and soak" function, which controls the temperature and its rate of change over a predetermined time span when more than one temperature is required to complete a process. Up to eight ramp and soak intervals can be programmed, each with a time duration of up to 254 repeat cycles. The CN-2010 has a multisetpoint function that allows presetting of up to nine independent setpoints. The controller accepts any standard thermocouple, RTD sensor, d.c. voltages up to 200 V or d.c. currents up to 200 mA. It will also interface with a host computer via an RS-232C, RS-422 or 20 mA current loop, allowing the user to access all input parameters and output values and assume direct control of outputs. *Circle No. 101 on Reader Service Card.*



The new stimulator from World Precision Instruments (left) and Newports' photodetector.

● Polypropylene disposable bags for autoclaving and sterilizing contaminated products are available from Sarstedt Inc. The bags are printed with prominent bio-hazard labels and come in three sizes. They can be autoclaved at up to 148°C.

Circle No. 108 on Reader Service Card.

● Tekspensor adjustable volume dispensers from Tekmar use micrometer adjustment for precise repeatability to dispense volumes from 0.4 to 100 ml with no mechanical slippage. Accuracy and reproducibility are better than $\pm 0.1\%$. The dispensers are made of chemically inert materials and the calibrated piston, suction tube and ejection tube are PTFE coated to minimize freezing problems associated with all-glass dispensers.

Circle No. 109 on Reader Service Card.

● The Bio-Rad Model 1305A variable wavelength UV monitor is a detector designed for chromatographic applications which require high sensitivity with low noise and drift. The monitor provides digital readout of absorbance, transmittance, or photo detector output independent of the recorder. The standard deuterium lamps allow a 190 to 350 nm wavelength range. The monitor may be upgraded by adding the optional tungsten lamp which extends the range from 350 to 600 nm. Sensitivity is selectable in eleven steps from 0.005 to 5.12 ABS/full scale. A time constant switch permits selection of a fast or slow response time. The monitor features separate electronic and optical modules to conserve bench space, and convenient interchangeable cassette-type flow cells for analytical, preparative, microbore, ultra-microbore and classical open column chromatography. The Model 1306 UV monitor is a simpler device for applications which require detection in the 190 to 350 nm wavelength only. The optical and the electronic components are located in the same unit, which makes this detector more compact than the Model 1305A.

Circle No. 110 on Reader Service Card.

● Chromatographic data can be acquired, analysed and reported using the new Adalab chromatography data system, Chromatochart and an Apple II computer. Data can be acquired from capillary and packed column gaschromatographs, HPLCs, gel scanners, TLC densitometers and amino acid analysers. It integrates and provides LC gradient control on four channels simultaneously, storing methods, raw data and reports on floppy disk.

Circle No. 111 on Reader Service Card.

● The new shaking waterbath from Haake Buchler Instruments Inc. is designed for gently agitating laboratory samples while holding them at a controlled temperature, as well as for specialized uses such as thawing frozen plasma or whole blood. The entire volume of the stainless steel bath is usable, since the heater and sensor are mounted below the bath. A large area heater reduces hot spots and provides even heating throughout the bath.

Circle No. 112 on Reader Service Card.



The Sarstedt disposable bag for waste (top) and Beckman's new pH meter.

● New from the National Appliance Company is a thermostatically-controlled waterbath capable of maintaining temperatures of 100°C when operated with or without a cover. Thermostatic controls allow use at lower temperatures, as well. A limiting thermostat protects the heater element from low-water conditions.

Circle No. 113 on Reader Service Card.

● Falcon Labware has introduced new 17 × 100mm round-bottom tubes for use in the collection processing, storage and centrifugation of biological specimens. The tubes have graduations for easy volumetric reference and are available fabricated from either polypropylene or polystyrene. The tubes are delivered sterile with a patented dual-position cap. The first position closes the tube but permits aerobic culturing of microbiological specimens. The second position fully seals the tube allowing it to be used for specimen storage and centrifugation.

Circle No. 114 on Reader Service Card.

● Standardized nucleic acid and immunological reagents for oncogene research are now obtainable from the North-London based company, Stratech Scientific Ltd. These highly purified, sequence specific DNA fragments have been designed for use in nick translation reactions and for the detection of oncogenic sequences by nucleic acid hybridization. Also available are antibodies for the detection of oncogene protein products and related antigens.

Circle No. 115 on Reader Service Card.

● Intended for routine and complex HPLC application, Gilson's new variable wavelength UV detector permits easy user programming of all instrument parameters. Operating from 190 to 380 nm, the Model 116 features a sensitivity range of 0.001-2.0 AUFS adjustable in increments of 0.0001 AUFS. The user can monitor at two different sensitivities simultaneously. Other features include a build-in keypad and computer interface.

Circle No. 116 on Reader Service Card.

● Human leukocyte interferon (α -IFN) intended specifically for AIDS research can now be obtained from Schwarz-Mann. It is prepared from normal human buffy coat cells induced by Sendai virus, purified by a modified Cantell method. The preparation is free of Sendai virus when assayed by haemagglutinin after serial passage through chicken eggs, and is free of hepatitis B surface antigen. Anti-human interferon α , purified human leukocyte interferon (α -IFN) and anti-human interferon β are also available.

Circle No. 117 on Reader Service Card.

● The Beckman Phi 40 bench-top pH meter is a simple-to-use instrument for accurate pH measurement in quality control, environmental and research laboratories. A custom-designed integrated chip gives the meter the sophistication of more costly models while making it easy to use. Most measurement steps are automated for nearly unattended measurement and simple operation. It has error and out-of-temperature-range warnings; always displays temperature; and does temperature computations with or without an automatic temperature compensation (ACT) probe. It features a digital-to-analog converter for simplified recorder setup.

Circle No. 118 on Reader Service Card.



The Bio-Rad 1305A (top) and Tekspensors from Tekmar.